RENAL FUNCTION

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Edited by

STANLEY E. BRADLEY
DEPARTMENT OF MEDICINE, COLLEGE OF PHYSICIANS & SURGEONS
COLUMBIA UNIVERSITY

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PARTICIPANTS

First Conference on Renal Function

MEMBERS

ROBERT F PITTS *Chairman*
Department of Physiology Cornell University Medical College
New York N Y

STANLEY E BRADLEY, *Secretary*
Department of Medicine College of Physicians & Surgeons Columbia University
New York, N Y

PHYLLIS A BOTT
Department of Physiological and Clinical Chemistry
Woman's Medical College of Pennsylvania
Philadelphia Pa.

DANIEL C DARROW
Department of Pediatrics Yale University School of Medicine
New Haven Conn

WILLIAM DOCK
Department of Medicine College of Medicine State University of
New York Medical Center at New York City
Brooklyn, N Y

ARTHUR M FISHBERG
Department of Medicine College of Medicine New York University
New York, N Y

ROY P FORSTER
Department of Zoology Dartmouth College
Hanover N H

ALLAN L. GRAFFLIN
Department of Anatomy School of Medicine Johns Hopkins University
Baltimore Md

CHARLES JANEWAY*
Department of Pediatrics, Harvard Medical School
Boston Mass

JOHN A LUETSCHER JR
Department of Medicine Stanford University School of Medicine
San Francisco Calif

JEAN OLIVER
Department of Pathology College of Medicine State University of
New York Medical Center at New York City
Brooklyn N Y

EWALD E SELKURT
Department of Physiology School of Medicine Western Reserve University
Cleveland, Ohio

JAMES A SHANNON
National Heart Institute National Institutes of Health U S Public Health Service
Bethesda Md

---

* Absent

NATHAN W SHOCK
Section on Gerontology National Heart Institute
National Institutes of Health and Baltimore City Hospitals
Baltimore Md

JOHN V TAGGART
Department of Medicine College of Physicians & Surgeons Columbia University
New York N Y

GEORGE W THORN*
Department of Medicine Harvard Medical School
Boston, Mass

GUESTS

ROBERT W BERLINER
Department of Medicine College of Physicians & Surgeons Columbia University
New York N Y

FRANCIS BINKLEY
Department of Pathology (Biochemistry), University of Utah College of Medicine
Salt Lake City Utah

ROBERT GAUNT
Department of Zoology Syracuse University
Syracuse N Y

HANS HELLER
Department of Pharmacology University of Bristol
Bristol England

LOUIS LEITER
Department of Medicine College of Physicians & Surgeons Columbia Univ
New York N Y

GILBERT H MUDGE
Department of Medicine College of Physicians & Surgeons Columbia Univer
New York N Y

HENRY A SCHROEDER
Department of Medicine The School of Medicine Washington University
St Louis Mo

JAMES V WARREN
Departments of Physiology and Medicine Emory University School of Medicine
Atlanta Ga

LAURENCE G WESSON, JR
Department of Physiology College of Medicine New York University
New York N Y

HARVEY LESTER WHITE
Department of Physiology The School of Medicine Washington University
St Louis Mo

A E WILHELMI
Department of Physiological Chemistry Yale University School of Medicine
New Haven Conn

JOSIAH MACY JR FOUNDATION

FRANK FREMONT SMITH, *Medical Director*
JANET FREED, *Assistant for Conference Program*

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* Absent

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1949 Conference on Renal Function

JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

FRANK FREMONT SMITH,
Medical Director

With the accelerating rate at which new knowledge is accumulating and with the increasing recognition that nature is of one piece it becomes evident that the continued isolation of the several branches of science from one another is a serious obstacle to scientific progress

Nowhere in science is the need for "combined operations" more evident than in medicine Today to be effective medical research and practice must embrace data from all the disciplines including nuclear physics at one end of the spectrum and cultural anthropology at the other for advances in one field are frequently dependent upon knowledge derived from quite another

Although the fertility of the multi-discipline approach is thus recognized universities scientific societies and journals have not yet made adequate provision for channels of interdisciplinary communication

I am delighted to welcome you to the first meeting of this group on Renal Function and to give you as briefly as possible an outline of what we hope to accomplish by these conferences

The Foundation is interested in furthering knowledge about renal function and is also interested in the broad aspects of problems of communication and integration which are important for the advancement of the whole of science It is our belief that scientific communications at scientific meetings and in the journals have been forced into a narrow mold in which logical sequence leading to inevitable conclusions is substituted for the much more flexible and often unpredictable processes by which scientific inquiry and the advance of knowledge actually take place All the creative the really exciting and interesting factors which are the soul and heart of science tend to be excluded today This unfortunately discourages individuals who have a creative and artistic turn of mind from entering the scientific field and creates in the minds of students and of the public a profound misunderstanding of the nature and processes of science

The Foundation s experience with the many research projects coming before it has led to the conviction that one of the greatest needs today is a re integration of science now artificially frag mented by the isolation of the several scientific disciplines and specialties Our Conference Program hopes to encourage this re-integration and to give in the published *transactions of our con ferences* a clearer reflection of what takes place in the laboratory and what goes on in the minds of investigators than now appears in scientific literature

Our eight groups on aging blood clotting and allied problems blood pressure biological antioxidants cybernetics infancy and childhood liver injury and metabolic interrelations (formerly meta bolic aspects of convalescence) which have been functioning for some years have held over 50 meetings in which more than 500 individuals have participated Recently five new conference groups have been organized on the following topics adrenal cortex renal function nerve impulse connective tissues and problems of consciousness

Each group will hold annual two day meetings for a period of five years It is our belief that only through continued association in an atmosphere of friendliness and mutual confidence can effective communication (exchange of ideas data methods and plans) across the barriers of the professions and specialties be promoted As a result of these meetings we have seen plans and ideas modi fied conclusions more clearly specified or placed in a broader perspective and spontaneous collaboration take place between investigators working in different departments or in different universities

As a nucleus 15 scientists comprise the original group of members for any conference These are selected by the Chairman of the Conference in consultation with the Foundation Every effort is made to include representatives from all pertinent disciplines From time to time new members are added by the group to fill gaps in viewpoint or technique A limited number of guests are invited to attend each meeting but for the purpose of promoting full participa tion of all members and guests attendance at any meeting is limited to 25

A point which I should like to stress before closing is that between the disciplines there are real difficulties in communica tion — partly emotional and partly semantic Emotionally some of us accept only data coming from those methods or disciplines with which we are familiar It is important that we do justice to the

validity of data and methods from other disciplines On the semantic level, the physical and biological sciences have little difficulty, the medical psychiatric and social sciences can understand each other fairly well, but to bridge the gap from the physical and biological sciences to the psychological and social is very difficult However, in the study of man all of the sciences must meet In medicine which must be equally concerned with the psychological and social as with the biological and physical there is the greatest opportunity, as well as necessity for mutual understanding among representatives of all the sciences I believe that the hope for a unification of science lies in the development of a *Science of Man* in which medicine must play a central role

In closing I want to say that the Conference Program is an experiment and that you are part of that experiment We hope that at these conferences you will feel the freedom inherent in the scientific method and will help us to improve our conference procedure

INTRODUCTORY REMARKS

ROBERT F PITTS, Chairman

Let me add to Dr Fremont-Smith's welcome my own very cordial welcome to you all Some of you I have met only in a very impersonal way through your scientific writings, it is a real pleasure to meet you here and to exchange views with you directly Many of you I welcome as old friends from whom I have in the past derived a great deal of inspiration

Basically we are gathered here because we have a common bond of interest in the kidney Some of us are biochemists or enzymologists, others are clinicians, and still others are morphologists I think we can find some common ground for free discussion in that we are all basically functionalists By drawing upon our respective disciplines, by freely exchanging our ideas, we can both learn from, and teach, each other

As the Chairman of this discussion group, I feel that the confidence which you have shown by appearing here at this conference session demands some justification for the program which has been arranged

The kidney by mutual agreement is a complex organ Yet there is no organ which can be studied functionally more completely in the intact organism We are indebted in large part to Dr Homer W Smith for the methods we use in such studies but we should not forget that we owe a great debt of gratitude to Dr A N Richards, Dr P B Rehberg Dr D D Van Slyke and others for fundamental contributions upon which these methods are based

Within recent years there has developed an increasing interest in kidney function for in what other field of human physiology are we so directly and urgently faced with the ultimate question, of how an organ as a whole functions and how the individual cells perform their respective tasks? Even on a clinical level there is an attempt to rationalize therapy in terms of the fundamental nature of the disturbances in cellular function

It seems to me, if I may be permitted a very hasty glance into my crystal ball, that we are on the threshold of major developments in renal cellular kinetics, and that in the not too distant future we may be able to look into the cell and understand what transpires there When we do, and when the rapid advances which are sure to

take place subsequently improve our understanding of the alterations of function in disease, we shall be a long way on the road towards more rational and more effective therapy

Because I am so convinced that the future of renal physiology lies in an understanding of cellular enzyme mechanisms, I have arranged a discussion of tubular absorption and secretion from the

in renal cellular function But I think we will all agree, that as one swallow does not make a summer neither does one enzyme make a functionally active renal tubular cell We shall, therefore, progress later to a somewhat broader discussion of the enzymatic aspects of tubular secretion and absorption We shall then consider to what extent we can fit these views into the challenging problems posed by tubular secretion of potassium and acid and by tubular absorption of chloride, sodium and water I personally am looking forward to learning a great deal from this conference we all shall I am sure if we air our views and discuss our problems frankly

Dr Oliver has very kindly consented to discuss from the functionally minded morphologist's point of view the problem of tubular secretion and absorption

1912) The dye is excreted in the urine and when he examined sections, not isolated nephrons, he saw that there was a taking up, or absorption if you will, of the dye particles in certain renal tubules He came to the conclusion from the study of his sections that it was the proximal convolution in which this change occurred Moreover, he described a gradient of absorption in the convolution which seemed quite reasonable, that is, most of the dye was taken up near the glomerulus and faded out as one went down the tubule These comments refer to an examination of the situation in histological sections, at best a subjective and difficult procedure Three years later his finds were confirmed objectively by an examination of nephrons which had been isolated by microdissection (Oliver J *The histogenesis of chronic uranium nephritis with especial reference* to epithelial regeneration *J Exp Med* 21, 425, (1915)) Such dissected nephrons are shown in Figure 1

On the extreme left is a nephron from a normal dog's kidney which was excreting trypan blue after intravenous injection and here one sees the typical picture of the gradient beginning with a considerable amount of blue dye absorbed into the tubule cells of the proximal convolution starting at the glomerulus (A) The amount of dye in the cells decreases so that about halfway down the proximal convolution no more dye can be seen Of course, if we looked with a higher magnification we would see some scattered down a little further but at no place else in the nephron are there *appreciable amounts of* trypan blue absorbed This particular process, as you all know, has been given a special name, *athrocytosis* I sometimes wonder if there is any need for setting off this process from some of the other mechanisms of tubular absorption Of course the absorption of a foreign body which is not metabolized *into the economy* of the organism certainly differs considerably from the absorption of something physiological which is actually used by the organism so that one would expect differences in appearances But after all it is absorption in both cases So, as I said I don't think this word athrocytosis is a happy one

Pitts I wonder if I could ask, for the sake of some of us, just what you mean by athrocytosis?

cases a foreign substance is used to demonstrate athrocyto[illegible] true that under physiological conditions some substances such as hemoglobin get into the tubule fluid They too are absorbed and

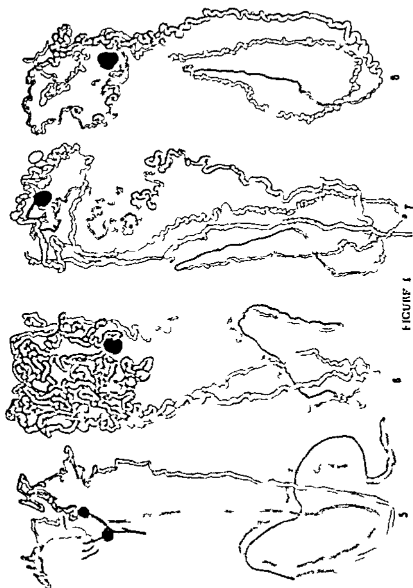


FIGURE 1

of course are metabolized within the renal cells and I presume the products of the metabolic change are either used or at least maintained in the economy Some would call this process athrocytosis because the drops of hemoglobin can be seen for a certain period after the actual excretion of the substance has ceased, perhaps a few days or so Then, as we shall see a little later, there are other substances in which the period of storage in the cell, athrocytosis if you will, is even more reduced There is therefore a continuing series of phenomena from the handling of a substance like trypan blue where the foreign body remains a long time stored in the cell, to a substance like egg white where it only remains a few hours One might continue the series to include amino acids which are absorbed and metabolized or passed on to the blood in a matter of minutes Electrolytes, I suppose, pass through the cells into the blood in seconds with little or no intracellular metabolic alteration Therefore it seems to me this special term athrocytosis, has a certain danger unless one understands exactly its significance

The other three nephrons on Figure 1 are from dogs that had a chronic canine Bright's disease as we can perhaps call it (Oliver, J Bloom, F, and MacDowell M Structural and functional transformations in the tubular epithelium of the dog's kidney in chronic *Bright's Disease and their relation to mechanisms of renal compensation and failure J Exp Med*, 73, 141 (1941)) It is a queer chronic interstitial nephritis which is quite different from glomerular nephritis or any other human disease, but it does have the same ultimate effect on the architecture of the nephrons as the diseases which one sees in man These nephritic dogs were in what one might call the compensated state of the disease although their kidneys were far from normal and the nephrons were functioning in a different way Still they were functioning and the kidneys as a whole were adequate physiologically Clinically, the dogs were not uremic Certain interesting points come out as one looks at different nephrons For instance the second nephron to the right in Figure 1 shows what extreme hypertrophy and hyperplasia can occur in the well preserved or less badly damaged nephron of the chronic renal lesion As I remember we actually measured this one I forget the exact figure but there is 15 or 20 times as much proximal convolution in this nephron as there is in the normal one on the extreme left, and as you see, it takes up the dye in exactly the same way Although it takes up much more, the dye does not extend any further down the convolution The localization or the relative localization at least remains the same I think this observation is

f some interest because skeptics have pointed out that large size
oes not imply a better functioning nephron This preparation shows
at they are better at least in handling trypan blue I will admit
at this may seem a rather unnecessary function perhaps to most
ogs but still it is evidence that a big proximal can do more than
small one The next two nephrons on the extreme right show the
hanges which occur when actual architectural differences have
eveloped You can see that the proximals are disarranged so that
here is a stretch of thin narrow tubule and at other places the
eneral configuration of the tubule has remained more or less
nchanged The interesting point is that where it has remained
nchanged morphologically its function so far as the trypan blue
s concerned also has remained unchanged The blue dye is present
ut in the atrophic parts of the proximal convolution there is no
ye at all These cells are not dead I want to make that point quite
lear – they are quite alive The only difference is that they behave
ifferently and they function differently Of course that leads to a
atter which has been a subject of some discussion and perhaps
ven controversy namely how should one interpret certain func
tional tests in abnormal kidneys which handle things differently
from normal kidneys

In kidneys in which the nephrons are actually damaged the

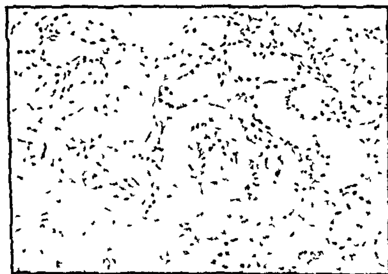


FIGURE 2

nephron that had been entered The kidney *was then taken* out and sent to us and we proceeded *to get the marked* nephron out Figure 4 shows the *piece of* kidney put into hydrochloric acid In (A) *you can see* that there is ink in one of the tubules That is the *one we went* after To the right above (B) you can see we are *dissecting* and isolating the nephron at the lower left (C) *the* nephron is isolated but the loops of its tubule are still entangled. *To the* right (D) it has been completely straightened out *We could* now make a camera lucida drawing and allowing *for foreshortening*, run a map tracer around it and so *make measurements We could actually find the hole* as indicated *by* the arrow in Figure 5 *just above* to the right where you see a break in the contour of the *tubule*, so that we knew where the pipet had entered Then making a camera lucida drawing we could determine exactly the source of *the sample*



FIGURE 5

Figure 6 shows typical findings The ratio between the concentrations of glucose in the tubular fluid and the plasma (TF/PL) was [illegible] dropped to 0 8 further down the [illegible] Finally it fell to 0 4 and to [illegible] in which nine tenths of the

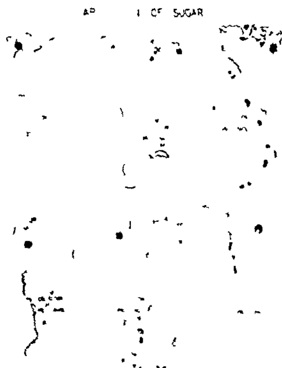


FIGURE 8

sugar had been removed, was obtained about halfway down the proximal convolution. This distance, because of the way the nephrons are placed in the kidney, is as far as one can reach with a pipet. If the animal had been given phlorhizin, the [illegible] sugar and the ratios as shown in Figure 7 rose. That [illegible]



FIGURE 7

ment was lucky because two samples were taken by chance from the same proximal tubule making possible two measurements of glucose ratios 1 6 and 2 0 Here the glucose concentration increased because the sugar was not absorbed and water was These findings permit calculation of water absorption Figure 8 shows the same facts expressed in a more conventional way In the normal animals the concentration of sugar falls to a very low figure and therefore the values for glucose ratio are less than one Following phlorhizin, the values rise

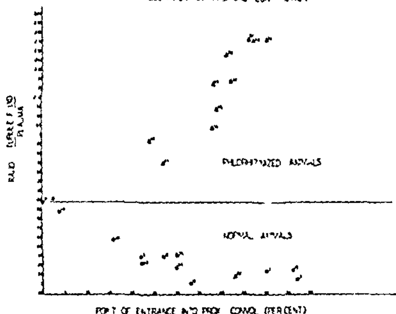


FIGURE 8

Here is another interesting point. If we look again at Figure 6 illustrating the normal absorption, we find that since glucose is practically all removed from the tubular fluid halfway down the proximal tubule, the terminal portion of the proximal convoluted tubule in the mammal comes in contact with only a very low concentration of sugar during normal life. In a human diabetic, of course, the situation is very different. Here glucose reabsorption is incomplete and so the terminal part of the proximal convolution and the remainder of the nephron come in contact with a considerable concentration of sugar perhaps for years. As you know, in the old days before diabetes was treated with insulin, glycogen was sometimes found in the kidney in remarkable amount (see Figure 9 (Oliver, J.: New directions in renal morphology. *The Harvey Lect.* (1944-45) 40, 102 (1945))). As in the section shown in the lower picture, the cells were so filled with glycogen that they had been transformed practically into nothing but huge globules of glycogen. This process can be localized in dissected nephrons as you see on the left. Here a proximal convolution is shown at a magnification of 150. We have to make illustrations of this kind



FIGURE 9

piecemeal because the long nephron won't fit under the microscope in one piece Probably thirty or forty negatives have been cut out and fitted together to make this illustration You can see the proximal convolution at the left in a sort of montage such as the astronomers use for making pictures of the Milky Way, or the Air Force in making maps Its first half looks entirely black That is because we preferred to stain it that way to bring out proper relative values What we are particularly interested in is the terminal part of the proximal convolution which is tremendously distorted since glycogen has filled the cells and turned the tubule into a

swollen lump In other words, the part of the proximal convolution which is conditioned to or, one might say is accustomed to the absorption of sugar takes care of it quite normally in the diabetic, but the portion of the proximal convolution which is not accustomed to absorbing sugar gets into difficulty A simple explanation — perhaps too simple — would be that the cells of the lower half of the proximal have absorbed the sugar and then have not been able to pass it on and that there then has been a synthesis of glycogen in the cell The fact remains however that the part of the proximal convolution which handles sugar normally does not get into trouble and the part that is not accustomed to handling sugar does

I would like expressions of opinion regarding any other explanation for such an intracellular cumulation of glycogen in the renal cells There is glycogen all through the nephron it is only this extreme concentration to which I am calling attention now Is there any more likely cause for an accumulation of glycogen in the cells than by absorption of sugar from the tubule fluid and synthesis of glycogen in the cell? Certainly the diabetic has little glycogen in his blood stream and so very little could be passed on to the tubule cell by either secretion or absorption It seems unlikely to me therefore that these accumulations can be of preformed glycogen Of course there is a general disturbance of the carbohydrate metabolism in diabetes and queer deposits of glycogen are found elsewhere so that some general disturbance may be entering into the production of this renal picture Still it seems to me that the most likely explanation of such extreme intracellular deposits in the renal tubule is a local synthesis of glycogen from glucose rather than absorption of preformed glycogen

White Does this deposition extend beyond the proximal tubule?

Oliver On the extreme right of Figure 9 the distal tubular convolution is shown In the upper part of this segment of the nephron there is never any accumulation of glycogen It is solid black in the Figure because its cells are normal Just below the solid black portion there is a light area where glycogen may be concentrated As I have said there is glycogen all through this nephron just as there is glycogen all through the liver and even the heart muscle but it is locally exaggerated in the proximal tubule

So much then for the localization of glucose absorption and glycogen synthesis So far everything has been absorbed from the tubule The only data that I have on the reverse form of transport passage from blood to tubule which I shall call "secretion" has to do again with an artificial dye neutral red There does not seem

to be much question as to the fact of secretion — Scheminsky showed these facts in Hoeber's laboratory a good many years ago (Scheminzky, F Uber die Harnbildung in der Froschniere XVII Die Farbstoffsekretion der 2 Abschnitte *Arch ges Physiol* 221, 641 (1929)) It just happened that at Stanford we were doing the same kind of work We had no idea they were working on this problem and within a month, I think the two papers appeared (Oliver, J and Shevky E A comparison of excretion of neutral red and phenol red by the frogs kidney *J Exp Med* 50, 15 (1929)) Later our conclusions were confirmed by Dr Richards by other types of experiment (Richards, A N Urine formation in amphibian kidney The Kidney in Health and Disease Chap 2, Philadelphia, 1935) In other words I believe it is quite firmly established that neutral red is secreted by the epithelium of the second part of the nephron of the frogs kidney, which is the analogue of the proximal convolution of the mammal We carried our work on a little further, bringing in the morphological aspect of this secretory process This extra vital method had certain advantages for it allowed us to do things one could not do with the older form of *in vivo* experimentation (Oliver J and Lund, E M Cellular mechanisms of renal secretion a study by the extra vital method I The Structural Phase II The Functional Phase *J Exp Med* 57, 435 (1933))

We studied the structural or morphological process of excretion of neutral red in the perfused kidney of the frog In that way we could eliminate a lot of complications We could perfuse the frog's kidney with a Locke's solution which had sugar in it and which either did or did not have neutral red We could also take out one kidney and look at it before we started the perfusion or before we put the neutral red into the Locke's solution, so we could se kidney not secreting neutral red and look at the other kidney whi had been secreting neutral red We found that the renal cells these kidneys were remarkably well preserved after this artifici perfusion with Locke's In fact, they looked exactly like physio logical kidney cells So what we then did was this We took out one kidney and looked at it as a control Then the other kidney of the frog was perfused with Locke's solution, in which there was no neutral red The Locke's solution had sugar that was all It was certain I think that there was nothing present that a kidney could secrete Then we could stain the cellular elements and see what had happened

Figure 10 shows what we saw in the first kidney before we started the perfusion Without going into too much technical detail,

it is stained with iron hematoxylin to show the rodlets. You can see the long filamentous batonets which fill the proximal convolution. Then the perfusion was started and after a certain length of time, perhaps half an hour, the kidney was fixed. The kidney had been functioning quite normally, that is, it had been absorbing



FIGURE 10

water and electrolytes and it had been absorbing sugar. When we looked at it, it looked for all practical purposes the same as the first unperfused kidney (Fig. 11). You can still see the rodlets. They are the long, filamentous objects.



FIGURE 11

Then we did another experiment in which we started out the same way. We perfused one kidney with the plain Locke's solution and sugar and here again you see what you have seen in the

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FIGURE 11

Then we did another experiment in which we started out the same way We perfused one kidney with the plain Locke's and sugar and here again you see what you have

previous two Figures, *that is, long filaments* (Fig 12) They lie on top of each other and they are not as clear as they would be if one could focus up and down, but they are certainly long filaments Then we put some neutral red into the Locke's solution and the kidneys began to secrete it as well as absorb the sugar, electrolyte, and water



FIGURE 12

After a certain length of time we took out that kidney and as you see it looks quite different in that the rods have disappeared (in most places at any rate) and are replaced by big drops (Fig 13) If one looks at these drops in fresh material they are red They have a large amount of dye in them But here is an interesting point If one isolates these drops they remain discrete in the Locke's fluid there is something in them that keeps them intact for they do not immediately dissolve and dissipate in solution If one prepares some of the isolated drops by crushing the tubule and treats them with Janus green the red drops turn a muddy green color In other words they take up the Janus green as well We will come back to the significance of this fact later, for the present we can assume that we have seen a change in intracellular elements, the rodlets, that occurs in the secretion of a substance, neutral red, and which does not occur in the absorption of water, electrolyte, and sugar We therefore suggest that these appearances are a part in some way of the mechanism of the secretion of neutral red.

More recently we have been interested in absorption and a situation, somewhat similar to that described for secretion of the dye, which seems to occur in the absorption of various proteins (Oliver, J The structure of the metabolic process in the nephron



FIGURE 13

The Edward Gamaliel Janeway Lecture *J Mt Sinai Hosp* 15, *175 (1948)*) This work was started during the war with a study of various blood substitutes, including gelatin and plasma proteins We thus obtained a lot of kidneys to study morphologically from Dr Addis who was studying the functional way in which the kidneys were handling these proteins

Figure 14 shows what we find when the serum protein of the rat has been raised considerably by injecting large amounts of

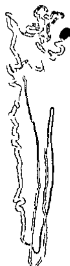


FIGURE 14

rat serum intravenously or intraperitoneally If this is done a pro teinuria develops If one gets out the nephrons as can be seen in figure 14 one finds intracellular droplets and these droplets show a very interesting localization They do not start out as one might expect in highest concentration near the glomerulus but there is a strip of tubule in which the absorption or the number of droplets is rather small and then in about the middle third of the proximal tubule the droplets begin to occur in great numbers and then again they peter out and disappear in the terminal part of the proximal convolution One does not see droplets in any cells elsewhere in the nephron

Figure 15 shows egg white droplets in rat tubule cells We did not use egg albumin but native egg white which is convenient because its proteins are of small molecular size and a lot of it comes through into the urine These droplets appear in the same part of the proximal convolution as the serum protein droplets If one injects enough this peculiar localization in the middle third dis appears The convolution begins to fill up and the distribution of



FIGURE 15







E 16

drops extends so that almost the whole proximal tubule becomes filled. The morphologist might naively believe that they are in part, at least, simply absorbed egg white. You will notice that where one sees many drops the rodlets seem to have disappeared, but this is shown much better in Figure 16 At the top is a higher magnification of normal rods fused together as a result of the staining technique. The other two photographs show places where drops are *forming and you see diffuse masses of material in the middle figure,* and definite round drops in the lower figure. It seems very obvious to a morphologist that the long rods have "dissolved." They have swollen and are disintegrating and one sees a lot of blackish material about them. Now if one stains the same kidney that you see in Figure 16 with Gram's stain, he gets a different and much clearer picture of the drops as shown in Figure 17, because the rods do not take up the Gram's stain. However, the drops are strongly Gram positive and you see them very clearly outlined in great numbers in the proximal convolution. The same stain can be applied to the isolated nephron and we find them in the middle third of the proximal convolution.

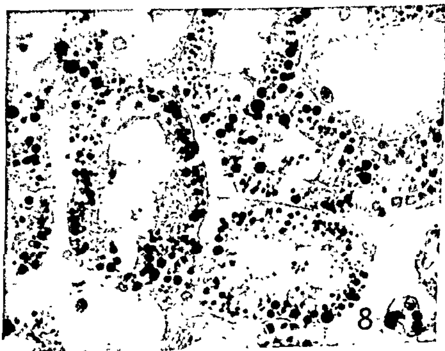


FIGURE 17

What is the significance of the fact that they are Gram positive? As you know the bacteriologists have looked into this matter and have by means of histochemical procedures involving the use of crystalline ribonuclease demonstrated to their satisfaction that the Gram positivity of certain bacteria is due to their content of ribonucleic acid so we did the same thing to the sections of these droplets (Fig 18) In the upper lefthand corner (A) is a section through a kidney field with egg white droplets stained with Gram s

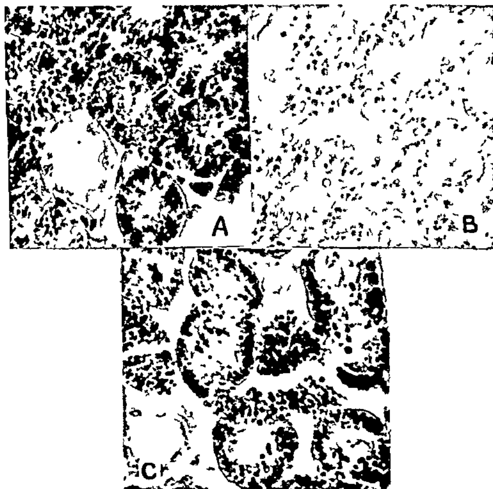


FIGURE 18

stain and you see the cells are filled with drops An adjoining section of the same block was put in a very low concentration of crystalline ribonuclease We used two preparations, Armour s and Worthington s, and obtained similar results The section is treated with the enzyme for an hour or so, washed and then stained with Gram and you see the remarkable difference But you will notice the drops are still there The ribonuclease has not digested them, it has simply more or less and in some instances completely, removed the staining affinity of the drop for the Gram's stain

Perhaps I should produce something here that might be called *prima facie* evidence At least it is temporary or provisional evidence that the Gram positivity depends upon the ribonucleic acid content in the drop There have been objections to such conclusions first, that the enzyme is not pure, that it has a mixture of protein dissolving enzymes in it, and also it has been claimed that although the enzyme may be pure, that the specificity of its action *is not* complete One way of answering these criticisms though I do not know that it is a complete answer, is to take a section such as (B) which is Gram negative, from which presumably the ribonucleic acid has been removed and place it in a solution of a salt of ribonucleic acid magnesium ribonucleic acid In other words try to put back what was taken out That is what we did and in (C) you see the droplets are just as Gram positive as they were before the enzyme acted upon them Whether this fortifies the argument that there is a considerable content of ribonucleic acid in these droplets or not I will have to leave to the enzymologists, but it seemed good evidence to us

I have not gone into the literature on this subject for it is a long story Other people have seen drops in renal cells particularly the Belgian morphologists, Gérard and Cordier, and in this country Smetana and others (Gérard, P and Cordier, R Esquisse, d'une histophysiologie comparee du rein des vertebres *Biol Rev 9, 110 (1934)*), Smetana, H and Johnson F R The origin of colloid and lipoid droplets in the epithelial cells of the renal tubule *Amer J Path* 109, *1029 (1942)*) They have examined the problem in different ways but I think it has been the assumption on everyone's part that the droplet was simply absorbed protein However, you see our experiment indicates that a very considerable part *of the* drop is not protein but ribonucleic acid, a fundamental element of

the protoplasm of the renal cells In other words the droplet seems to be a sort of coacervate or combination of protoplasmic elements and the absorbed egg white

One can perhaps get at this problem more exactly if one grinds up the kidney and prepares a suspension of the nuclei red blood cells and all the intracellular elements including the filamentous mitochondria and the droplets Such procedures have become standard methods with biochemical cytologists One can prepare suspensions in sucrose or saline solution although there are advantages in using sucrose All this must be done at a low temperature so that

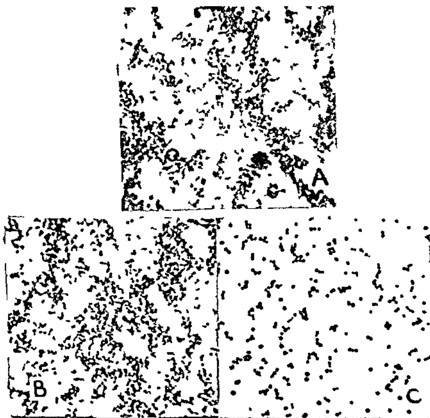


FIGURE 19

That there is a further metabolism and degradation of the egg vhite, even the morphologist can show What I have been describng is the intracellular status 17 hours after injection Examinaion of Gram stained sections from kidneys at varying time in ervals after the injection of egg white shows what might be lescribed as a cycle of change in the droplets At three hours they ire few in number, small and only weakly Gram positive At 17 iours as I have described, they are large, deeply Gram positive and o numerous as to fill and distend the cell body At this time an)ccasional droplet can be found which either stains less strongly or is physically disintegrating as is evident by the broken contours of the droplet The intracellular "digestion' of the droplets pro ceeds so that after 72 hours only a few persist Strangely enough, these few scattered droplets may be present for weeks

In other words, we have morphological evidence of the fact that the egg white is being acted upon by enzymatic processes within the cell and that it is being broken down It therefore is not surprising that at 17 hours we found only five percent of native or original egg white What we are doing at the present time is [illegible] in the earlier [illegible] we can thus [illegible] ietabolism of the egg white within the droplets in the renal cell There is just one further tubular process which I wish to consider from the morphological viewpoint I cannot say whether it is absorption' or 'secre tion', only that it is an increase in the concentration of mineral salts in the cells of the nephron One can study this process very accurately by getting the nephrons out on a slide and incinerating them and so destroying everything but nonvolatile minerals The ash settles down on and adheres to the slide and produces the so called spodogram This is a replica of the original structure of the tissue which has been incinerated One can increase the mineral content of the kidney by giving calciferol and parathormone Whether this increase is evidence of an "absorption" or "secretion" or just simply a "deposit" in the renal epithelium I don't know but at least there is something about the process which you can see This is the specific localization shown in Figure 20 (a) These are spodograms from a normal rat To the right is the blood vessel which is curving around and from it come afferents on which are glomerular tufts, *the round objects There is not very much mineral in the* vessel or in the afferents which are therefore difficult to see Then below is a glomerulus and the first part of a proximal convolution

enzymes wont change everything One can then separate the particles into fairly pure fractions by repeated centrifugalizations In this way the drops may be separated from the rodlets or mitochondria There is a supernatant left which contains what are usually called microsomes We have gone no further in trying to separate these intracellular particles

Some examples of our preparations are shown in Figure 19 Above (A) is a mixture of everything You can see short filamentous particles which are mitochondria and round drops that are considerably larger Also you see debris and large objects that are probably red blood cells Below the suspensions are fairly pure At the right (B) is a relatively pure suspension of mitochondria though there are a few round objects that may be droplets Certainly the suspension of droplets on the left side (C) is I think remarkably pure

Grafflin This is in sucrose?

Oliver Yes The procedure is simply an adaptation of other people s methods Hogeboom Schneider and Pallade were the men who established the particular methods we are using (Hogeboom G H Schneider W C and Pallade G E Cytochemical Studies of Mammalian Tissues 1 Isolation of intact mitochondria from rat liver *J Biol Chem 172 619 (1948)*) After the suspensions were prepared our biochemist Dr Werner Straus analyzed them

Our interpretation of the biochemical analyses of the particulates is that the intracellular droplet at 17 hours consists of a small amount of egg white determined by immunological methods and a considerable amount of something resembling the mitochondrial substance of the rodlets in its lipid phosphorus and ribonucleic acid phosphorus This would seem to support the morphological suggestion that the drop is not a drop of egg white but that it is composed of cytoplasmic elements (mitochondria or rodlet material) and absorbed protein

Such a conclusion is interesting because it complicates the whole matter It also leads to a possible extension of our knowledge of just what is going on in the renal cell when it absorbs protein It has been shown by others that certain of the enzymes are concentrated in the mitochondria One might suppose therefore that what we are seeing in this combination of cytoplasmic material (mitochondria) and egg white is the bringing together of the substrate (egg white) and the enzyme and that the droplet formation is the mechanism by which the two operate in the intracellular metabolism of the absorbed egg white

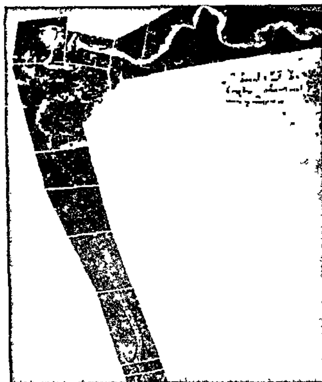


FIGURE 20(b)

words there has been an increased concentration of nonvolatile minerals in the proximal convolution after the administration of calciferol Looking at the ascending limb and the distal below it can be seen that this is not the case There is no more than there

in it that one can hardly see its outline So here again there seems to be a definite localizat on of a metabolic process that can be seen by '

parathormone is required to produce damage It is an interesting point that if one stains these kidneys in which the nephrons con tain several times their normal calcium or total mineral ash by the

and here the minerals are more *heavily deposited I think that the* irregularity is the result of our maceration We have to soak these kidneys in hydrochloric acid in order to dissect them and this takes out a large amount of the mineral We have actually determined how much is taken out and under our particular method of maceration about nine tenths of the total mineral is removed by the preliminary treatment with acid So this Figure shows only a part of the original total This is probably fortunate for if it were all present the preparation would be so dense and heavy that no detail would be discernible It is clearly seen that the proximal on the *right has much more mineral in it than the ascending* limb and *distal which is upside down and in part almost invisible* from its low ash content on the left



FIGURE 20(a)

If one gives an animal calciferol (or parathormone) and increases *the mineral excretion (Fig 20 (b))* one observes a very definite change in appearances an overall change but the same sort of relative distribution At the top is the proximal convolution and in *it one sees what one might call* plates or caked solid masses of mineral ash Most of it of course is calcium but other elements are increased too It is quite different from that first proximal In other

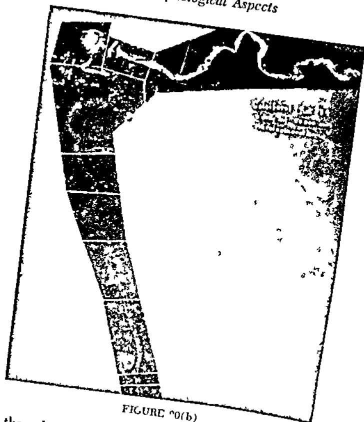


FIGURE 20(b)

words there has been an increased concentration of nonvolatile minerals in the proximal convolution after the administration of calciferol Looking at the ascending limb and the distal below it can be seen that this is not the case There is no more than there was in the normal control animal Beginning below is the ascending limb One has difficulty following the course of the tubule as it gets up toward the distal convolution because there is so little mineral in it that one can hardly see its outline So here again there seems to be a definite localization of a metabolic process that can be seen by the morphologist

Pitts What would happen with prolonged treatment?

Oliver Damage in the kidney results Much more calciferol than parathormone is required to produce damage It is an interesting point that if one stains these kidneys in which the nephrons contain several times their normal calcium or total mineral ash by the

conventional methods which *the pathologist* employs to demonstrate *what he calls* calcification one can see *no increase* in calcium at all There is no calcification in the old sense of the word I think therefore that here we have found a method of studying what might be called the preliminary stages of calcification before the actual deposit in its obvious histological form occurs

Schroeder Can you tell where it is in the glomerulus?

Oliver In the tuft We have some photographs in which one can see the tuft of the glomerulus quite clearly with its lobulations and in it the concentration is high

These then are the examples which I wanted to show in which a morphologist can at least point and say "Here something is happening Often he cannot say much more than this at times by crude histochemical procedures he can guess but his hope is that now the biochemist and enzymologist can step in and say what really is happening in these places where structure has been disturbed

DISCUSSION

Shannon It might be helpful for Dr Grafflin to comment on some work he did several years ago His work on the cellular dif

Grafflin It has been adequately demonstrated that the proximal tubule (i e the entire portion of the nephron provided with brush border extending from the glomerulus to the transition to the thin segment) of the cat and dog nephron characteristically contains fat under normal conditions and that this fat is regularly distributed in a particular manner in the two species (Peter K Die Nieren

Epithelzellen der Saugetierniere *Arch f mikros Anat* (1911) Nakamura S Beobachtungen uber die Fette in den Harnkanälchen von Katze und Hund *Folia Anat Japon* 13 45 (1935)) In the cat the entire *pars convoluta* and the upper portion of the *pars recta* are normally fat laden while the terminal portion of the *pars recta* is fat free In the dog the reverse is true the fat being confined exclusively to the terminal portion of the *pars recta*

In tubules teased from acid maceration material the transition between the fat laden and fat free portions is characteristically abrupt (Peter Nakamura *loc cit*) Zimmermann added greatly to our knowledge by demonstrating that the abrupt transition with respect to fat content is likewise abrupt with respect to cell type In both species the luminal cell boundary pattern in the terminal portion of the *pars recta* – fat free in the cat fat laden in the dog – is rectilinear In the remainder of the proximal tubule – fat laden in the cat fat free in the dog – the luminal cell boundary pattern is very complicated showing in marked degree the interdigitations usually considered characteristic of the mammalian proximal tubule Despite the reversal in fat distribution in the two species these findings seem to justify the conclusion that we are dealing here with two distinct segments of the proximal tubule Whether the bulk of the proximal tubule (exclusive of the terminal portion of the *pars recta*) can be further subdivided on cytological grounds must await further work As is well known Suzuki has subdivided the mammalian proximal tubule into three or in the rabbit four segments (Suzuki T Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen Jena *(1912)*) but no critical cytological proof of his thesis has yet been adduced Since in both cat and dog the abrupt shift in fat distribution demarcates so accurately the cytological transition between the upper and lower segments Foote and I have used isolated proximal tubules stained for fat as a means of obtaining quantitative measurements of the relative lengths of the two segments (Foote J J and Grafflin A L Quantitative measurements of the fat laden and fat free segments of the proximal tubule in the nephron of the cat and dog *Anat Rec* 72 *169 (1938)*) From each species sixty complete proximal tubules – fifteen from each of four animals – have been isolated from maceration material and measured In percentage of the total length of the proximal tubule the average length of the second segment is 19 5% in the cat 10 8% in the dog

Oliver In the cat we are quite certain that the gradient of fat deposit in the cells of the proximal convolution is not as it is in trypan blue The latter is an even gradient which is high at the glomerulus decreasing gradually downwards But with fat there is something rather similar to what is seen in the absorption of the protein It is first moderate reaches a peak in the mid proximal and then fades out as can be seen in Figure 21

Grafflin The distribution of fat in the proximal tubule of the cat nephron was found to be relatively constant The typical f ,



FIGURE 21

may be summarized as follows At the glomerular end the fat begins abruptly with no apparent neck segment *The fat droplets here* are of both small and medium size and are quite numerous For a short distance distally along the tubule they gradually increase in size their numbers remaining essentially the same After the fat deposit has become heavy it remains so until near the distal end of the first segment, where the fat gradually diminishes At the end of the first segment the fat stops abruptly and completely, the second segment is wholly fat free

If we drop back to the fishes and limit the discussion to four representative species with which I am personally well acquainted the proximal tubule in the aglomerular toadfish (*Opsanus tau marine teleost*) exhibits only one cell type, whereas the proximal tubule in the glomerular sculpin (*Myoxocephalus octodecimspinosus* marine teleost) eel (*Anguilla rostrata* euryhaline teleost) and lung fish (*Lepidosiren paradoxa* fresh water) shows a differentiation into two histologically distinct segments The question arises as to what fundamental homologies if any, exist between these differentiated portions in the four representative nephrons under considera

tion In the sculpin and eel it seems perfectly clear on histological grounds that the lightly staining first portions of the proximal tubules in these two species constitute one homology and that the deeply staining more highly organized second portions constitute a second homology Furthermore, there can be no doubt that the proximal tubule (of one cell type) in the toadfish nephron is homologous with the second portions of this segment in the sculpin and eel and is markedly dissimilar in structure to the first portions in the latter two species If we attempt to extend these homologies to include the lungfish the problem is more complicated In *Lepidosiren* the order of the lightly staining and deeply staining portions (speaking in very general terms) of the proximal tubule compared with the situation in the sculpin and eel is reversed the deeply staining portion following directly upon the neck segment Histologically and cytologically the structure of the second portion is so striking and unique that it most emphatically is not homologous with either of the differentiated portions of the proximal tubule in the sculpin and eel With respect to the first portion in *Lepidosiren* admittedly shows certain distinctive features not encountered in the nephrons of teleosts Nevertheless if any fundamental homology exists and I believe that it does this first portion is homologous with the second portion of the proximal tubule in the sculpin eel (Grafflin A L The structure of the nephron in fishes *Anat Rec 68 287 (1937)* this paper includes references to the important work of J G Edwards and other investigators in this field)

The proximal tubule in the kidneys of amphibia and birds has not been adequately investigated with respect to segmental differentiation In the mammals we have the very sharp differentiation of the proximal tubule into two segments in the cat and dog which we have already discussed and the available evidence indicates that the proximal tubule in the rat is probably subdivided into at least two distinct segments Edwards has reported that the injection of iron salts into the rat leads to a deposition of iron exclusively in the terminal third of the proximal tubule (Edwards J G Functional sites and morphological differentiation in the renal tubule *Anat Rec 55 343 (1933)*) In a group of five rats which exhibited a striking deposition of yellow to golden brown pigment in the cells of the proximal tubule the distribution of the pigment was studied in complete proximal tubules isolated by teasing after maceration with hydrochloric acid The major portion of the proximal tubule beginning at the glomerulus exhibited considerable variations in the amount and character of the pigment but these differences

were always achieved more or less gradually, with no suggestion of sharp intersegmental transitions On the other hand, in the majority of the tubules studied the terminal portion of the proximal tubule was set off from the remainder of the proximal tubule by a rather abrupt alteration in pigment deposition and, in addition, by the presence of isolated pigment-laden epithelial cells The average length of this terminal segment was approximately 25 percent of the total length of the proximal tubule (Grafflin A L The storage and distribution of iron containing pigment and the problem of segmental differentiation in the proximal tubule of the rat nephron *Am J Anat*, 70, 399 (1942))

Adequate cytological evidence for such segmental differentiation of the proximal tubule has never been supplied for the other laboratory mammals or for man

Shannon My question was prompted by Dr Oliver's comment about the deposition of glycogen in cells of the lower part of the proximal tubule when exposed to glucose He suggests that such an abnormal accumulation of glycogen occurs because the cells in the lower portion of the proximal segment are not normally exposed to an environment of glucose on their tubular lumen side He also suggests that glycogen does not occur in the more proximal portions of the segment since these cells are normally exposed to glucose It seems more likely to me that there is a functional differentiation within the proximal segment and that the abnormal accumulation of glycogen under Dr Oliver's particular experimental condition results from the different functional potentialities of the cells in the area concerned rather than from the circumstance that they are under his conditions, first being exposed to an abnormal environment This is merely to emphasize that in the teased total nephron one cannot consider the proximal segment to be made up of cells that are wholly homogeneous The difference in morphology certainly carries with it a suggestion of a difference in functional potentialities

Grafflin I should like to ask Dr Oliver whether he sees a very sharp transition in the proximal tubules of kidneys exhibiting heavy deposits of glycogen

Oliver Oh, yes We have dissected literally dozens of glycogen kidneys and they are all just the same one is the dead ringer for the next except in the amount of degree of swelling The tubule goes along quite even and straight and then suddenly swells where the glycogen has been deposited

Shannon Anatomical appearances may vary radically under different conditions quite independently of functional characteristics Most people looking at the histological preparation of the brush border for example have the conception that it is just what it looks like that it is a stiff brush On the other hand it is my understanding that it is a very mobile area in life capable of striking cellular activity

Grafflin The brush border is a fascinating structure and can give highly variable pictures depending upon the fixatives employed The more brutal acid fixatives are most likely to produce the picture of discrete hair like processes suggested by the term "brush" By the proper choice of fixatives one can obtain almost any variant between this extreme picture and that of a smooth almost homogeneous border with little or no evidence of vertical striation Some years ago I made some studies of brush border in fresh spreads of the mammalian kidney and frequently I was able to follow the gradual development of striations in borders which were optically structureless when they first came under observation Wislocki and Bennett have reported some very pertinent observations upon the variable morphology and mobility of brush border in the placenta of man and monkeys (Wislocki G B and Bennett H S The histology and cytology of the human and monkey placenta with special reference to the trophoblast *Am J Anat* 73, 335 (1943)) Twelve years ago in Copenhagen Dr Wigglesworth demonstrated to me some almost incredible pictures of mobility in the brush like border of the nephric tubules in an insect *Rhodnius prolixus*

Oliver We have looked at these borders in rat kidneys with the phase microscope in the fresh or living cell suspended in serum I do not know how quickly we looked at them that of course is a good point since their structure may vary with time — but as soon as we did look which was within a minute or so they seemed to be definitely brush like more like hairs The thing that astounded me was their appearance in the isolated cell that is in a cell that had been completely isolated from its habitat and suspended in plasma or in Locke's solution The cell is quite round and the brush border is a halo or crown around it that sticks out in every direction It looks much like the corona of the sun in total eclipse It gives one a completely different picture than a microscopic section where the brush borders are stripes on the top of the cells The phase microscope might be a way of working with it

Shannon Just one other point I would like to recall some observations made by Robert Chambers some years ago He

Wilhelmi 10 000 to 16 000

Oliver Such as the basic proteins protamines for example? The trouble with them is that they are very toxic

Wilhelmi Another one not toxic is cytochrome C which you could use

Oliver Could we see it in the cells?

Wilhelmi You can see it spectrophotometrically because it has a nice nucleus for absorption

Schroeder Unfortunately it does not stay in the kidney as cyto chrome C There has been some work done by Beinert et al with cytochrome containing radioactive iron and the iron is found in the ferritin fraction in the kidney and liver (Beinert H Maier Leibnitz H Richey E O Reissmann K Further investigation on the injection of respiratory catalysts in an attempt to improve anoxia toler ance *School of Aviation Medicine Quarterly Research Report 22 (July 1 to September 30 1949)*

Wilhelmi After you give cytochrome C?

Schroeder It very rapidly disappears A considerable amount is excreted in the urine and the rest of it is apparently changed much of the iron being taken over into the ferritin fraction

Oliver I know nothing about it As to the preparation of cyto chrome C can one prepare a considerable amount easily?

Binkley You can buy it easily

Oliver What is its size?

Wilhelmi Between 10 and 16 000 so it would be roughly one half to one third the size of albumin

Oliver It might in its pattern of absorption resemble more closely trypan blue

Bott I still have some of that PPD protein that Dr Florence Siebert let us take which seemed to be about 85 percent permeable and that has a molecular weight of about 14 000 I do not know how you can detect it but it does have many characteristics of a com plete protein

Oliver It is really the larger particles that I would like to try something large rather than small

Bott Did I understand Dr Oliver that you think the glomerular membranes of the rats kidney are more permeable to serum pro tein than those of other mammals?

Oliver That is my impression and it is based on work done by Dr Addis and by Dr Gilson (Gilson S B Studies on proteinuria in the rat *Proc Soc Exp Biol and Med 72 609 (1949)*) I do not think there is any question that serum albumin for instance goes

through the rat glomerulus because the rat has a proteinuria all the time

Berliner Whipple and his co workers (Terry R Hawkins D R Church E H and Whipple G H Proteinuria related to hyper proteinemia in dogs following plasma given parenterally *J Exp Med 87, 561 (1948)*) have found that if a normal dog is injected with large doses of dog plasma a marked degree of albuminuria occurs when the plasma protein is sufficiently elevated

Oliver I have never seen morphological evidence that globulin is absorbed by the tubule hence it should appear in the urine if it is filtered

Bott Samples of fluid taken directly from the tubules in mammals by Dr Walker (Walker A M Bott P A Oliver J and MacDowell M C The collection and analysis of fluid from single nephrons of the mammalian kidney *Am J Physiol 134 580 (1941)*) occasionally contained some protein but many samples contained none by the method used – about 30 mg percent is the lowest detectable concentration

Oliver That would mean a lot of protein in the urine by the time it gets to the bladder?

Bott Yes

Oliver It would be a lot in the urine if none were absorbed in passing down the tubule The whole problem – this fact that we have the glomerulus putting protein in and the tubule taking it out and our not knowing the relative degree of either – is very confusing

White Has anyone ever tried to detect the presence of protein by any method other than just the cloud formed on adding a protein precipitant?

Bott Dr Hudson working with Dr Walker and Dr Richards (Hudson C L and Mudd S An ultramicro technic for precipitation and agglutination reactions *J Immunol 28 34 (1935)*) used an immunological method which was very promising but so far as I know it has not been used since I think it should be reinvestigated especially in the light of some of the recent developments Dr Richards and I carried out a series of perfusion experiments some of which were made on necturi We made glomerular punctures during the perfusion of various protein solutions and used a modification of the Folin and Ciocalteu method really Greenberg's protein method We were not able to measure much lower concentrations however than with the simple test But it was useful for these quantitative comparisons of perfusion fluid with glomerular

fluid (Bott P A and Richards A N The passage of protein molecules through the glomerular membranes *J Biol Chem* 141, 291 (1941))

Wesson Is it possible to interpret some of these morphological gradients down the proximal tubule which Dr Oliver and Dr Grafflin have described on a functional basis? What I have in mind is this The tubular fluid in one portion of the nephron will have a different composition from the fluid in another portion Perhaps the *differing appearance of the cells represents the response to differing* compositions of the tubular fluid Both the concentration and the mass of any particular substance in the tubular fluid may increase remain the same or decrease as the fluid moves down the tubule In the case of colloids there may be a tendency for the tubular fluid to separate out particles of different sizes in different regions according to the physical properties of the flow In the case of proteins it might be possible that the rate of accumulation by the cells is some function of the concentration of protein in the tubular fluid

Oliver In the case of trypan blue that is the way I had rather vaguely thought of the process That is the amount available falls as the substance is absorbed and therefore one naturally expects an evenly falling gradient but when one considers those substances that show first a low absorption a peak of absorption further down and then a drop as the proteins do then I do not know how to explain the gradient on simple physical factors of relative concentration The rate of absorption of water is faster than the rate of absorption of protein and therefore the protein becomes more concentrated and therefore more is in contact with the cells so they take up more You can think of various possibilities which get terrifically complicated I do not know any way of measuring the factors involved

Pitts I suppose change in ionic environment *might change* the physical properties

Oliver I may say with all due deference to my fellow morphologist *Dr Grafflin that though I have never had* any doubt about the differences in cell pattern I have not thought of the problem from this angle alone I have thought the gradient as due probably to unknown physical conditions As the fluid goes down the tubule the ionic pattern changes and this might cause great physical differences in the state of the protein which is to be absorbed The *change in ionic pattern might* be either more or less favorable to cell activities so that under some conditions they would absorb

more and others less Without trying to go into it, I had always thought of this sort of mechanism rather than that there were different cells that had a different job to do

Pitts I wonder if in this connection Dr Forster might not want to comment on the neutral red story

Forster Yes, by way perhaps of forcing a definition out of Dr Shannon or some others of the word secretion" I would like to refer back to Dr Olivers clean cut experiment in which he perfused the frogs kidneys first with Lockes solution and then removed one of the kidneys for histological study The remaining kidney was perfused with Lockes solution plus neutral red for a period of time and then also examined histologically So then he had two kidneys one of which had secreted neutral red and the other one which had not secreted neutral red nor any other kind of substance Then as you remember he pointed out that as a concomitant of the secretion of neutral red there was a change in the nature of the cytoplasmic structures The filamentous structures changed over into droplets or at least the droplets were present when a substance was being secreted and those droplets were not present in the absence of secretion

The mechanism of transport or secretion" if we can use the word of neutral red is quite unlike the transport of such substances as phenol red (phenolsulphonephthalein) diodrast p amino hippuric acid and those compounds which we ordinarily use for measurement of tubular function Neutral red is transferred only as long as the urine is more acid than the fluid on the other side of the tubule cells Furthermore neutral red continues to be "secreted" by cells even after those cells have been treated by such metabolic poisons as cyanide whereas such exposure to cyanide or other inhibitors blocks the secretion of phenol red (phenolsulphonephthalein) As long as the urine is acid the movement of neutral red from the peritubular fluid into the lumen can be observed even in the presence of metabolic inhibitors A common laboratory experiment in general physiology which I believe, was suggested originally by Chambers points up again the need for a definition of this phenomenon which I don't think can be called secretion He showed that an excised frogs bladder containing acid urine tied up to form a sac and dropped into an alkaline solution containing neutral red will "secrete" neutral red across the inert membrane until a high concentration of the dye is achieved

Another difference between neutral red on one hand and such other substances as p amino hippuric acid diodrast and phenol

red, on the other, is that the former stains the more acid vacuoles within the cell and becomes concentrated in intracellular droplets This is not the case with phenol red As an isolated renal tubule preparation, fish tubules may be placed in a balanced isotonic salt solution containing one milligram percent of phenol red Direct observation of these tubules *in vitro* reveals that phenol red is secreted until a concentration of dye is achieved in the lumen of as high as 100 milligrams percent Phenol red moves against a considerable concentration gradient, irrespective of the pH of the urine without at any time an intracellular accumulation of dye becoming apparent

I wonder whether we can make a distinction at this time between these two mechanisms of cellular transport as exemplified by the movement of neutral red on one hand, and by the passage of phenol red, p-amino hippuric acid, penicillin diodrast, etc, on the other?

Oliver I am sure that neutral red is a very special case I think everything that you have just mentioned is more or less implicit in some early work we did For instance, the effect of acid on its excretion we considered before Dr Chambers and Dr Kempton became interested in the problem (Oliver J and Lund E M Cellular mechanisms of renal secretion II The Functional Phase *J Exp Med* 57, 459 (1933), Chambers, R and Kempton R T: The elimination of neutral red by the frog's kidney *J Cell and Comp Physiol* 10, 199 (1937)) We tried a few perfusion experiments with phenol red and abandoned them just because phenol red did not behave in the same way as neutral red Moreover we could not see the things that we could see with neutral red and being morphologists we wanted to use something that we could see

I remember an amusing interchange of letters with Dr Chambers during his work on the problem of the effect of acidity on the excretion of neutral red, in which we both wondered what "secretion" was and when we should use the word and when we should not My attitude has always been that I did not know and therefore I did not like to set up some particular "thing in itself" called "secretion" as different from other forms of cellular passage I think I suspected something vitalistic about the term "secretion" and therefore I just didn't like it No doubt I acted from pure prejudice

You can even extend this sort of argument to include terms like "absorption" All these things go together so that when we begin talking about "secretion," "absorption" and about some "metabolic process" in the cell, we are making very artificial distinctions In our

protein work we used to think of it as a study of "absorption" Now we are thinking of it more as a study of "intracellular metabolism", that is the degradation of protein What we originally thought of as an indication of "absorption" we are beginning to believe may not have so much to do with the entrance of material into the cell but rather with intracellular metabolic processes

Pitts Don't you have some pertinent data on those points Dr Shannon?

Shannon It may be worthwhile to emphasize at this point that the type of transport mechanism utilized in the transference of neutral red across the cell is similar to that used by a number of weak organic bases where apparently movement across the cell is determined largely by the dissociation constant of the base This was demonstrated by Jailer and others (Jailer J W Rosenfeld M and Shannon J A Influence of orally administered alkali and acid on renal excretion of quinacrine and chloroquine and santoquine *J Clin Investigation 26 1168 (1947)* Jailer J W Zubrod C G Rosenfeld M and Shannon J A Effect of acidosis and anoxia on concentration of quinacrine and chloroquine in blood *J Pharmacol & Exper Therap 92, 345 (1948)*) In these cases secretion" or "reabsorption appeared to be wholly determined by the hydrogen ion concentration of the urine

Furthermore it was possible to demonstrate that such a phenomenon was not a distinguishing characteristic of renal tubular cells but actually could be demonstrated in the body as a whole This is important since apparently one is dealing here with a very general phenomenon which to my mind has little to do with active tubular transport or tubular excretion In the case of neutral red I believe it advisable to view the total phenomenon as consisting of two discrete events one of these is concerned with the movement of neutral red across cell membranes The other is concerned with what happens to the neutral red once it has entered the cell When neutral red moves across the renal tubular cells under certain circumstances it does at the same time behave in a very peculiar manner relative to its localization in the cell That fact would appear to be a coincidence rather than evidence that one phenomenon is wholly dependent upon the other Some evidence for this view stems from the toxicity studies on 4-aminoquinolines which behave relative to renal transport mechanisms in a manner quite similar to neutral red These antimalarials were administered to a variety of hosts in very high dosage actually to the point of death the animals being subsequently studied for evidence of

toxicity Morphologic studies of the kidneys of these animals revealed little in the way of information which would indicate that incidental to the transport of these substances across cell walls abnormal accumulations occurred in the cells themselves

In general my feeling is that it is quite important to differentiate between what we have felt is a non specific movement of material across cells and the biological mechanisms which are available within the renal tubular cell to produce diffusion gradients at the expense of energy These latter are commonly called secretory or transport processes

Oliver What would be some of the *bona fide* secretory activities in contrast to those observed in the handling of neutral red?

Shannon Phenol red is a good one A great deal of work has been done with it As Dr Forster brought out, there are apparently sharp gradients under the brush border because phenol red does not stain or concentrate in cells

Oliver I am not quite sure of that I have the recollection – it has been a long time – that we did see droplets of phenol red in some of our perfused kidneys

Shannon You do if you use very high concentrations but not in the ordinary concentration

Oliver These were in perfused kidneys

Shannon In the chick mesonephric preparations of Chambers, one does not find any degree of concentration of phenol red in the cells at ordinary concentration However, it is my recollection that when Dr Chambers went to such high concentration as 100 mg percent, there would occur cellular inclusions rather deeply stained Dr Chambers took these as an indication of toxicity of the phenol red to the cells The lack of such an occurrence at lower concentration was taken to indicate that these are not an essential part of the transport mechanism

Oliver Of course the morphologist does not like to be told that as soon as he sees something things are abnormal, although I will admit that this at times may be the case

Bott Dr Shannon, do you make the same distinction between reabsorption of glucose and the so called reabsorption of protein? I don't feel that they are the same at all

Shannon I feel that the reabsorption of glucose is quite different from the reabsorption of protein wholly apart from the difference in physicochemical characteristics of the two substances The cytological study of cells during an active secretory process on exposure of a renal cell to protein solution is difficult since the control kidneys

or the control cells are not in a truly resting state Consequently it is not logical to conclude that the transport of protein creates a cytological picture which is specifically related to an actively secret ing cell as opposed to a resting cell The tubular cells in the control of kidney are far from resting since they are actively doing work with expenditure of energy in the reabsorption of salts and other solubles I suspect that when one imposes an abnormal variable the cytological picture must be a reflection of the cell s reaction to that specific variable and the morphologic change with the protein is not one which can be interpreted to indicate the morphologic change which is to be expected with a variety of transport processes as contrasted to what is present in the rest of the cell

Oliver As I understand it then any morphological change which is seen is *per se* abnormal?

Shannon You have changed the normal economy of the cell

Oliver That leaves the morphologist in the situation where he is not much use

Shannon No sir I would not say that It leaves the morphologist in the position where he has to carry that thought in mind in interpretation

Oliver We have all that in mind

Fremont Smith Abnormal with respect to what? With respect to the problem you are dealing with? It seems to me that a change can be looked upon either as a normal or abnormal change of function

Shannon The point I wish to emphasize is that the so called

it can be used as a control but not as a so called resting control It is difficult for me to see actually how one can obtain an adequate control so as to isolate one functional characteristic from all other variables

Fremont Smith You feel that the control should have been the normal resting cell?

Shannon To which you don t have access

Oliver No cell is "resting except in death Both morphologists and functionalists must accept living cells as active and use them as "controls" under appropriately similar conditions

Heller It may be difficult to arrive at a picture of resting cells in the frog s proximal tubule even when all exogenous substances

which are known to be secreted are excluded There is good evidence in a paper by Marshall (Marshall E K Jr The secretion of urea in the frog *J Cell Comp Physiol* 2 349 (1932)) that the frogs kidney secretes urea Your control kidney presumably was secreting urea all the time?

Oliver Only that urea which might have been brought to it by the Lockes solution washing through tissue I would agree with Dr Shannon fully What he describes is the ideal procedure for both the morphologist or the functionalist but it does not seem to me it is compatible with any realistic activity on the part of an investigator

I have had a tremendous boost from Dr Opies cytological work of the last few years He has taken a cue I think from the biochemist Instead of trying to preserve tissues perfectly to fix them exactly without change he deliberately rips them to pieces by letting them macerate in fluids of various sorts In other words he does just what the biochemist does when he begins boiling leaching precipitating and what not No one doubts that by this method the biochemists have learned something They do not try to handle tissues or their problem in a physiological way They do quite the contrary They disrupt things and find out artificial" facts yes but from those artificial facts they can perhaps go back to physiological conditions I think the experimental morphologist is in the same situation When he uses something like neutral red that is obviously a foreign material he is under no illusion that it bears any immediate identity to the handling of some ideal physiological substance but at least he is finding out something

Fremont Smith The physicists taught us when they tried to measure speed and mass of the electrons that you cannot observe any phenomena in nature without changing them — because by virtue of observation you are changing them If we accept that premise then our job is to specify the nature of change we induce by observation and how it affects the interpretation of observation

Shannon That is a perfectly valid point I think we do have an experimental approach to such a problem If one examines a series of other transport mechanisms such as glucose phenol red diodrast or the like one way or another and if one examines the cytology of the particular part of the kidney involved at very low concentrations of a perfusing fluid and at very high concentrations where the mechanism is saturated one then has a controlled observation that has more validity for comparative purposes than when a completely new variable is superimposed upon a series of unknowns Presum

ably the kidney can operate on these highly specific transport mechanisms without fundamental disturbance of many of the normal characteristics common to the cell

Leiter Dr Shannon, what is the approximate size of neutral red and does the size have any bearing on the type of transport process?

Shannon It is the same order of magnitude as that of phenol red However, it does undergo a varying degree of dissociation

Oliver The size depends upon pH One can observe change in size As it becomes more acid or alkaline one can see this with the ultramicroscope

Shannon It tends to form aggregates

Leiter Does it reach the size of inulin?

Oliver No We do not have absolute measurements but I do not think so

Schroeder Does the normal kidney ever secrete anything that normally occurs in the body at usual concentrations?

Shannon Ammonia

Fishberg That is synthesized in the kidney but does the kidney tubule transport anything normally circulating in the blood?

Pitts Presumably it would transport hippuric acid

Fishberg Is any of the urinary hippuric acid derived from outside of the kidney?

Binkley Yes

Berliner Maybe somebody is enough a chemist to say whether or not this is a valid concept I wonder if the difference between secretion and the type of thing we have with neutral red and atabrine, etc might not be expressed as follows any process which results in the transfer of a substance from a position where its activity is low to one where it is high might be defined as secretion (By activity here I mean chemical potential or escaping tendency) On the other hand a transfer which does not involve a change in the activity of the substance would not be called secretion The transfer of neutral red would fall in the latter category The activity of the free neutral red may be the same in the plasma and urine and the difference attributable to the amount of the salt present in one phase and in the other I am not certain of the physical chemistry involved Somebody else might do better with it

Pitts Dr Wilhelmi will you take a crack at that?

Wilhelmi It seems a reasonable point I am not enough of a physical chemist either to say positively that would be the case Certainly one could argue for ions that if there is an increase in activity in one compartment greater than in another one would

expect work to be done and you could describe that with a little elaboration as involving an active principle There are as we know from Donnan effects instances in which one can end with different concentrations of ions provided that you begin with the system at disequilibrium and allow it to come to equilibrium I think that this need not apply here since it is difficult to imagine that an organ like the kidney might arrive at a condition like equilibrium in physical chemistry

Forster I would like to get back if I may to the question which was raised before whether materials in the course of transport across the renal tubules accumulate in the cells or not Dr Oliver made the point that perhaps neutral red did not differ from phenol red in this regard In the passage of neutral red not only does one note accumulation of dye in the lumen but also in the intracellular droplets which become intensely stained This is a colored photograph (Frontispiece) of an isolated renal tubule taken from a fish *and placed in a balanced isotonic salt solution which had oxygen* bubbling through it and contained a one milligram percent concentration of phenol red It is apparent that the cells of the renal tubule have not taken up any of the red dye Inasmuch as the protoplasm is slightly alkaline one would expect that the phenol red should stain the cell contents if the dye were present in any significant amount The dark line indicates the accumulation of phenol red in the lumen which in this instance approached a concentration one hundred times as great as that in the fluid bathing the tubule

I think you showed Dr Oliver and I know Chambers did that phenol red accumulated in the cells only when instead of one milligram percent or so the *in vitro* solution contained dye in concentrations of the order of twenty five milligrams percent The situation where dye is taken up in high concentrations intracellularly is quite different from that which prevails with the transport of phenol red at low plasma levels We have reason to believe that the mechanism involved in the latter is the same as concerned with the transport of diodrast p amino hippuric acid and the other substances customarily used for renal plasma flow measurements This is indicated by the observation that the addition to the *in vitro* solution of substances that compete for transport such as caronamide penicillin diodrast and p amino hippuric acid will block the transport of phenol red Presumably this dye is carried across the tubules without accumulation in the cells and its transport is facilitated by some expenditure of metabolic energy on the part of the cells There are two basic differences between this transport mechanism and that

operating in the movement of neutral red First neutral red accumulates within the cell a fact which might account to a great extent for the transformation of filamentous structures into droplets The droplets may actually represent concentrations of neutral red The second point of difference is that the movement of neutral red does not involve the expenditure of energy So I think the situation which you described as a concomitant of transport of neutral red that is the droplet formation is a very special case Didn t you say that in some of your experiments you actually had observed phenol red secretion and noticed that you did not get the cytoplasmic transformations?

Oliver That is true and I also should have said that we used various concentrations and times of perfusion of neutral red I have thought about this neutral red work for so long that it comes a surprise that there is still any interest in it but I remember that made a very sharp distinction relative to the passage of neutral from the capillary to the lumen in the perfusion experiment which in a simple minded way we called "secretion meaning only it was going in that direction nothing more) between what called *direct* secretion and *indirect* In the early stages of any perfusion experiment one gets considerable amounts of neutral red passing into the tubular lumen without any drops forming in the cells and this passage can be affected by certain elecrolytes As I recall calcium slowed or decreased the passage Then as opposed to this or added to it was an *indirect* method of passage in which we observed accumulation of the dye in droplets in the cells So neutral red certainly can pass through the tubule wall without causing droplets It commonly does in any perfusion experiment and then as time goes on accumulation begins and this other method proceeds I made quite a point of what we called direct secretion Perhaps it is not a good point We might have said *immediate* and *delayed* secretion the first having nothing to do with droplets and the second involving the appearance of droplets

Shannon It might be helpful were the phenomenon examined in tissues other than kidney Perhaps Dr Grafflin would be willing to comment on the behavior of macrophages in neutral red solutions more particularly in relation to the second type that is the intracellular type of handling this material by a nonrenal as well as a renal cell

Grafflin The simplest preparations for the study of this problem are fresh spreads of subcutaneous connective tissue which has been injected with a dilute solution of neutral red In such preparations

the macrophages routinely segregate the dye in vacuoles or droplets which at least in the majority of instances, so far as one can tell, do not pre-exist in the cell The size and number of these vacuoles tends to increase with time, and many of them are very highly colored indeed

Oliver By "segregate' you mean "concentrate'?

Grafflin Yes

Shannon This would appear to be an excellent example of what [illegible] d occurring in [illegible] would appear [illegible] portant to ap preciate *this and to appreciate further that when the phenomenon* does occur in a cell capable of active secretion, the phenomenon itself is not necessarily related to the latter function

Oliver These general facts do not make the situation different It simply means that what we are dealing with is the very fundamental process that living cells have of absorbing neutral red What happens then depends upon what the cell is The kidney cell passes it on to the tubule lumen

THE ROLE OF GLUTAMINASE IN TUBULAR PROCESSES[1]

FRANCIS BINK
Departments of Pathology and Medic
University of Utah College of Medici

Before I start on the discussion of my topic[2] I would like to brin up something that Dr Wilhelmi and I have been discussing. In regard to the problem of glycogen deposition there is a possibility that the cells in the upper part of the tubules have the hexokinase system The glucose is phosphorylated by this reaction to give glucose 6 phosphate this reaction is thought to be the one blocked by phlorhizin and the one concerned with the reabsorption of glucose If this reaction is blocked then the glucose will pass down to the cells in the lower tubule and may be absorbed as glucose 1 phosphate a precursor of glycogen

My interests are not distinctly in renal physiology but are concerned with the general processes of absorption by all tissues We have been driven to this concept by our studies of hereditary diseases including muscular dystrophy Several theories concerning the creatinuria of dystrophy are possible Either creatine is produced in great amounts so it is coming out in the urine or it is produced in normal amounts and is not absorbed or retained by the muscle tissue Therefore we have labeled creatine with heavy isotopes and have administered it to patients We find that within 48 hours all the administered creatine has been excreted In other words the dystrophic muscle is unable to absorb or hold exogeneous creatine presented to it So our problem would appear to be simplified All we have to do is to determine how the muscle normally absorbs its creatine through its membranes and how it holds it

For some time I have been working on the metabolism of glutathione and glutamine We had found that an enzyme of the kidney hydrolyzed glutathione at a very rapid rate so that the glutathione was broken down to an amino acid glutamic acid plus the dipeptide cysteinylglycine (1) Since there was so little known about the meta

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[1] From the Laboratory for the Study of Hereditary and Metabolic Disorders

[2] These studies were supported by grants from the U S Public Health Service K Nakamura J Watson C A Olson and J R Kimmel have collaborated in the investigation of various aspects of the problem

bolism of glutathione we thought it would be of some interest to isolate this enzyme from the kidney and study its behavior We are finally convinced that we have the enzyme in fairly pure form The enzyme is active in the hydrolysis of glutamine to glutamic acid plus ammonia As you are aware, this is the mechanism which has been suggested for the formation of urinary ammonia as reported by Van Slyke (2) But on the other hand this enzyme appears to constitute a very great part of the protein of the kidney The material which we isolate is a lipoprotein which by the criteria we can apply to it appears to be homogeneous It is not soluble enough to permit studies in the electrophoresis apparatus The studies in the ultracentrifuge indicate a molecular weight of ab two million The nitrogen content of this material is about 1 percent It contains approximately 25 percent phospholipid Tl material constitutes 30 percent of the protein of a homogenate kidney tissue

It had been our conclusion that this was a magnesium activatec enzyme but when we ran studies of activity of the enzyme as a function of the concentration of magnesium ion it was found that at concentrations of 0 001 M magnesium there was activation As the magnesium ion was increased to or near 0 01 M, complete inhibition was observed

Immediately we turned to a study of the effects of a large number of ions (Fig 22) The large number of the ionized materials of importance in renal physiology can be divided roughly into four groups In one group like urea and creatinine concentrations may be increased to 3 000 mEq per liter with no effect upon the activity of the enzyme Then we have another group represented by glycine and containing most of the amino acids There is an activation that approaches a maximum no further change is observed as the concentration is increased Another group represented by lithium chloride or mercuric chloride was found to inactivate the enzyme when present in small traces Then another large group of materials represented by sodium chloride was found with these materials a biphasic phenomenon was observed Activation was observed up to a certain concentration and then inhibition occurred Sodium sulfate behaved roughly like urea and creatinine Sodium bicarbonate and potassium chloride have presented much the same behavior as sodium chloride

We have tested a group of the so-called ... and have found them to be ... (Fig 23) One of the po

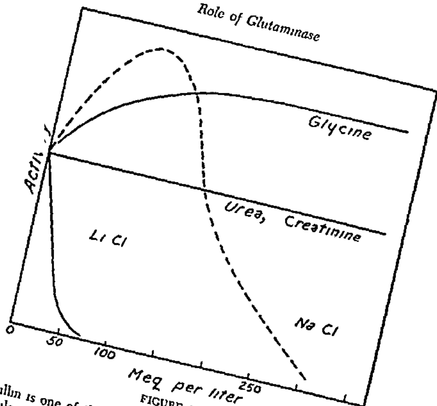


FIGURE 22

Penicillin is one of the best Phenol red, diodrast, carinamide, and sulfanilamide were found to be competitive inhibitors We turned then to a study of quite a few of the various phenolphthaleins and sulfonphthaleins We find that these dyes inhibit, as competitive inhibitors only when the pH of our buffer is greater than their pK For instance, phenol red is an excellent inhibitor of glutaminase at about 7.4 but phenolphthalein has no effect In fact it activates slightly as the pH is raised to about 8.5 at the higher pH it becomes a potent inhibitor

There are some interesting effects that I can describe only in general terms For instance if this enzyme has the importance that I want to ascribe to it you may well expect that marked hormonal effects should be observed We have found that thyroxin in more or less physiological concentrations is an activator of the enzyme Activations as great as 200-300 percent have been observed If however, the concentration of thyroxin is increased far beyond the physiological range, inhibition is observed.

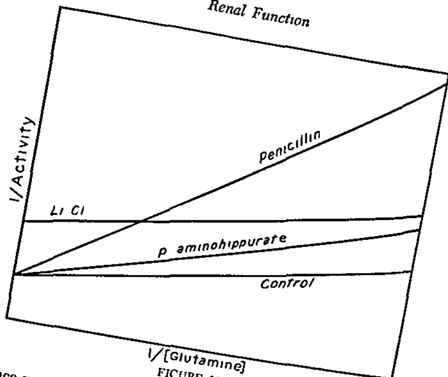


FIGURE 23

Since we are interested in absorption mechanisms in all tissues as well as in the kidney we have studied the enzyme in other tissues We find that the intestine has a system identical in so far as we can tell with that of the kidney It hydrolyzes glutamine and glutathione and like the kidney enzyme is inhibited much in the same manner The phthaleins that are excellent laxatives at the p of the intestine are good competitive inhibitors of the enzyme a higher values of pH Those that are not laxatives are not inhibitors

The same system is not found in all tissues We feel we have two types of glutaminase the brain type and the kidney type The kidney type is found in the intestine but the nervous tissue type or brain type is found in muscle and all nervous tissue In the brain type the activity toward glutamine is much greater than the activity toward glutathione whereas in the kidney and intestinal type they are about equal

It is my thesis that this enzyme is a logical candidate as the mechanism for certain major aspects of absorption by the kidney We do not intend to imply that if the immediate source of energy for absorption is the hydrolysis of glutamine and glutathione such processes as the formation of hydrogen ion in metabolism or phos

phorylation are not of importance On the other hand we feel that we can explain a lot of renal function if we are granted that this insoluble lipoprotein behaves as a self regenerating ion exchange resin Certain of the reactions are illustrated below

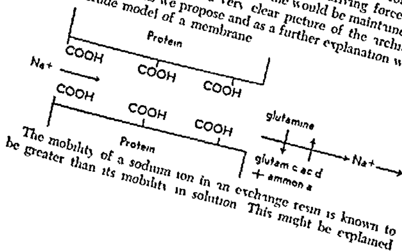
Absorption of Sodium

(1) Protein – COOH + NaCl $\rightleftharpoons$ Protein – COONa + HCl

(2) Protein – COONa + glutamine $\rightleftharpoons$ Protein COONH$_4$ + glutamic acid

(3) Protein – COONH$_4$ + glutamic acid + energy $\rightleftharpoons$ Protein – COOH + glutamine

In these reactions which are analogous to those that take place with a resin of the carboxylic acid type the sodium is removed from solution by the resin even though the equilibrium is in the opposite direction All that needs to be done is to lower the concentration of sodium ion at some site within the cell on the other side of the membrane and thus trick the sodium ions into moving into this area In other words there must be a reaction in which sodium ions are shoved off this is the reaction which we ascribe to glutaminase activity Hydrogen ion might be expected to play a role in this process since it is a cation that can be produced by the oxidation of substrates However since the ammonium ion can be produced and withdrawn under physiological conditions and without demands upon buffering mechanisms it would appear to be the more logical substrate The removal of the sodium ion is pictured as its replacement by ammonia As the ammonia is resynthesized into glutamine a resin carboxyl is freed for further combination with sodium In this manner a driving force for the pumping of sodium across the membrane would be maintained This explanation does not give a very clear picture of the architecture of such a system as we propose and as a further explanation we propose a crude model of a membrane



The mobility of a sodium ion in an exchange resin is known to be greater than its mobility in solution This might be explained

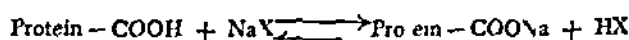
by a migration of the sodium ion from carboxyl to carboxyl grouping

We have spoken of the system as one that requires glutamine as a source of energy It is quite possible however, that amide groupings are formed on the protein and glutamine is involved only as a donor of amide groupings to the protein In fact, such a mechanism is suggested by our observations that there is a loading of the enzyme molecule with amide groupings during the course of enzymatic activity

It is obvious that a major consideration in any such mechanism is the architecture of the cell It is unfortunate that the kidney is so completely unidirectional in its activity for such behavior imposes many restrictions on any model that may be proposed

Acidification of the Urine

Pitts has shown that acidification of the urine can be explained (8), by known reactions of the type of resin we are proposing as the absorptive unit When a salt of a strong base and a weak acid reacts with a carboxylic acid resin, the equilibrium lies well toward the left



$$\text{Protein}-COOH + NaX \rightleftharpoons \text{Protein}-COONa + HX$$

It would be expected that sodium or potassium salts of acetate or pyruvate, or disodium phosphate would take part in such reactions

Formation of Urinary Ammonia

It is characteristic of carboxylic acid resins, such as IRC-50 that ammonia may be displaced by hydrogen ions in the range of pH developed by the reactions described above for the acidification of urine It is difficult to visualize how penicillin and phenol red are transported across a membrane I frankly don't know, but what I can do is to give a suggestion For instance we have found that an inhibitor must have three qualifications (a) It must be an anion It must be capable of being an anion at the pH of the buffer used most of them contain carboxylic acid groups (b) It must contain some type of resonating system and (c) it must contain a large R group For instance the phthaleins are inhibitors Such things as bicarbonate, acetate propionate and pyruvate are activators but as the higher acids are reached they become indifferent and eventually became weak inhibitors That is an observation which is slightly reminiscent of some work that has been done in the organic chemistry of the amide groupings Steinhardt has shown that a large group of materials aid or catalyze the hydrolysis of amide groupings (4) It has been suggested that amide groupings combine with these anionic

reagents to give an amide salt Perhaps we can assume that the agents for measuring renal blood flow or inhibitors have actually combined with the amide groupings of the protein or glutamine I am inclined to believe that the enzyme is the one with which it combines We have evidence that this is true because we can show that glutaminase will take up phenol red Phenol red attached to the glutaminase can be shown to be displaced from the enzyme by the addition of glutamine Thus it appears that the phenol red is combining with exactly the same groupings that combine with glutamine Since such agents do catalyze the hydrolysis of amide groupings this is possibly the mechanism by which they are carried through as the anions Some adaptation of the concept is undoubtedly necessary but that is our explanation as it stands at this time

You may have gathered from this presentation that I feel that a kidney is essentially a tube of ion exchange resin and that the stratification or localization of absorption which is observed is due 'o the effect of one ion upon the other There is no real reason for ssuming that these cells are different in their absorptive behavior If a mixture of ions very much like that found in the glomerular filtrate, is run through a column of IRC-50 a very similar stratification of these various ions is observed

There is one more point I would like to consider We started in with this program by worrying about the behavior of creatine Unfortunately creatine has very little effect upon this enzyme Creatine phosphate however behaves very much like glycine Of some interest to us is that the glutaminase of muscle is associated in the actomyosin complex In so far as we can determine it is the actin of actomyosin that has the glutaminase activity

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DISCUSSION

Mudge Would you tell us how you prepare this enzyme?

Binkley Our preparation is from pig kidney It need not be fresh but can be stored frozen One hundred grams of pig kidney plus

1 000 ml of 0 01 M sodium barbiturate pH 11 is homogenized and heated at 45° for two hours This is centrifuged at 6 000 for twenty minutes Then the supernatant is dialyzed against distilled water for 24 hours The distilled water is changed about every four or five hours Then we pass in CO for a long time The enzymatically active material is precipitated As we purify it it becomes more insoluble We start with 100 grams of kidney and end up with the enzyme in two liters of 0 01 M sodium carbonate bicarbonate mixture of pH of about 8 5 The next step is alcohol fractionation If we fractionate at this stage without changing the ionic strength the active material is precipitated between 3/10 and 6/10 volumes of ethanol In other words to two liters we would add 600 cc centrifuge discard that material and add another 600 cc. and centrifuge

We have varied ionic strength many times for further fractionation If the material is dialyzed exhaustively one may add three volumes of ethanol and centrifuge to get rid of inactive material then pass in CO to precipitate the active material We lose at most about 10 to 15 percent activity when treated with acetone and stored in the desiccator it is stable for months

Leiter Have you extracted renal cortex and medulla separately?

Binkley We have tried to We have not made much progress

White Do you have any observations on red cells say of man where sodium is pumped out and potassium in?

Binkley That is about the only species on which we have any information This brings up one important point In the red blood cell glutathione predominates and in the plasma glutamine predominates As you know ammonia given to an animal disappears very quickly If ammonia is added to ordinary oxygenated blood the ammonia disappears with the formation of glutamine and at the same time a decrease of glutathione It appears that the mechanism used to pick up the ammonia the glutaminase is in the cell stroma If we take the stroma through an alkaline extraction we find the enzyme in solution

White It would be interesting to know whether the mechanism is present in red cells which do not establish concentration gradients of sodium and potassium as well as in those that do

Taggart One is impressed with the fact that this 30 percent value is fairly close to that which Schneider (Schneider W C Intracellular Distribution of Enzymes III The oxidation of octanoic acid by rat liver fractions *J Biol Chem* 176 259 (1948)) has reported for the combined nuclear and mitochondrial fractions of

liver and kidney cells The mitochondrial fraction of cell particles apparently contains all of the enzymes which are needed to implement the reactions of Krebs tricarboxylic acid cycle and a number of others besides Green (Green D E Loomis W F and Auerbach V H Studies on the Cyclophorase System I The complete oxidation of pyruvic acid to carbon dioxide and water *J Biol Chem* 172, 389 (1948)) applied the name "cyclophorase system" to this integrated complex of enzymes Is it not conceivable that your glutaminase lies within such a complex and that the rather rigorous conditions of preparation have effectively destroyed the other more labile activities?

Binkley I believe that is possible

Taggart Yet you have spoken of the enzyme as being homogeneous

Binkley I say "complex I won t say pure substance" in the sense of a protein

Taggart When one uses the term complex" a certain lack of homogeneity is often implied You will agree then that the glutaminase may be just one of many enzymatic activities originally present in the complex one which has survived the method of preparation

Binkley That is why I was interested in Dr Olivers comments I hope to get at that by the use of staining materials Our enzyme stains quite beautifully by something like phenol red

Schroeder Is this enzyme specific for glutamine? Does it attack histamine or histadine?

Binkley It also attacks glutathione but has no activity on histamine or histadine

Schroeder What about other primary and secondary amines as sources of ammonia?

Binkley I have not tried them This enzyme is concerned with the hydrolysis of a peptide bond There are lots of things about the metabolism of glutamine and glutathione that puzzled people for a long time Every one has talked about glutathione as a possible activator of enzymes yet it has a turnover out of all order of magnitude for such an activator Certainly even from that data one might conclude that the biological utilization of glutathione is concerned with the hydrolysis

In passing I would like to mention that brain slices with the addition of glutamine will retain potassium Another very important thing that Krebs and his group have found is that a few toxic thyroids have very low glutathione values We have confirmed that

This is in line with the activation of the hydrolysis of the glutamine by thyroxin

Other work that may be explained partially on the basis of our theories is the work of Conn and his group on the effect of ACTH on the lowering of the level of glutathionine in blood We believe that the ACTH stimulates an increased utilization of glutathione for the pumping mechanism, probably to such a point that it is depleted and glycosuria develops The amount of glutathione normally supplied is not adequate to keep up with the load and more glutathione must be supplied as fuel for the pump

You would probably be interested as to whether or not we have made any calculations as to the energy required by this mechanism, whether we can explain, for instance, within the observed oxygen consumption whether or not the kidney can use this as a pumping mechanism It can but not with too great a degree of efficiency

Taggart What is the approximate efficiency required?

Binkley About 30 percent

Taggart Do you believe that the glutaminase catalyzes both the synthesis and hydrolysis of glutamine?

Binkley There is evidence that this is so

Taggart How do your views on glutamine synthesis compare with those of Speck? (Speck, J F The enzymatic synthesis of glutamine, a reaction utilizing adenosine triphosphate *J Biol Chem*, *179, 1405 (1949)*)

Binkley My work is not in opposition to that of Speck It is a little bit different You will see that it is probably the same story We found if we lowered the pH down to about 7 in the hydrolysis of glutamine, the hydrolysis stopped rather abruptly at an equilibrium point Instead of glutamic acid, pyrrolidonecarboxylic acid, the anhydride of the glutamic acid was formed Pyrrolidonecarboxylic acid with our enzyme, plus ammonia, gives glutamine, that is to say that the hydrolytic enzyme also catalyzes the reverse reaction

What Speck has shown is that adenosine triphosphate plus glutamic acid and ammonia gave glutamine We find that pyrrolidonecarboxylic acid plus ammonia gives glutamine Apparently his system contains an additional enzyme which is responsible for the formation of pyrrolidonecarboxylic acid from glutamic acid

Wilhelmi Do you think that the activating effect of the amino acid, such as glycine, is due to its removing metallic ions?

Binkley Glycine has a very great effect, histamine has practically no effect The amino acid story is fairly complex I don't know why

some have such effects and some not Undoubtedly it has to do with the combination of other ions with the amino acids

Pitts What is the effect of calcium salts?

Binkley At low concentration activation at high concentration inhibition

Schroeder Would you say something about the effect of potassium?

Binkley If we get our enzyme free of sodium as we can by constant dialysis against potassium buffers then the enzyme is inactive It requires sodium ion to be active and potassium does not replace the sodium Potassium chloride does have an effect more or less like sodium chloride in the presence of sodium ions

Schroeder Krebs in a recent symposium shows that shaking up a piece of muscle in a solution results in loss of potassium from muscle at a constant rate If glutamate is added to it it does not. If you added glutamate to the solution after the potassium had left the cells it went back into the muscle The concentration of the glutamate was so small that it could not have been due to simple ion exchange It was more like an active process

Binkley The way we interpret the function of glutamine in the muscle is that the muscle is utilizing glutamine to pump out sodium and the potassium comes in to replace the sodium The pump is reasonably indifferent to potassium but not completely so

Taggart Does phosphate have any effect?

Binkley I will have to explain several things about our phosphate effects You may recall that Dr Greenstein has been working with glutaminases He has described glutaminases I and II I is activated by pyruvate and II is activated by phosphate His evidence for calling them I and II is that the one activated by phosphate has a different pH optimum than the one activated by pyruvate and the activation is different in character The activation by phosphate shows a cut-off value whereas that by pyruvate does not We have made the same observation with our purified materials We think the glutaminases are identical the effect of phosphate is to shift the activity down towards neutrality Since we are working with the sodium salts of weak acids in both cases we may consider an exchange type of reaction If the phosphate concentration is too high the exchange will be stopped by inhibition of the enzymatic step

Pitts What is the difference in the *in vitro* behavior of the enzyme to penicillin versus carinamide say with the addition of glutamine?

Binkley Not too different Penicillin is just a much more potent

inhibitor than carinamide, that is really the only test we have made

Mudge Have you tried mercury or tetrathionate?

Binkley Mercury is very potent as an inhibitor We have not tried the other

Heller Is the system inhibited by sulfanilamide?

Binkley Yes

Shannon I was wondering in calculating this as an ion exchange resin what the capacity of the resin is for sodium It is terribly important to calculate capacity You are trying to use a non specific ion exchange resin as a model to explain differences in the renal handling of specific ions If the capacity is as large as you suggest it should not differentiate between the sodium and potassium ions I believe it is only when the resin begins to reach the point of saturation that one may expect a differential action on the uptake of specific ions with such a resin

Binkley I agree there are certain important things to be considered in a quantitative manner When I was showing you the effects of the ions on the enzymes (Fig 22), it was mentioned that the cut off values corresponded to the 'threshold' values If we adjust our enzyme and the pH we can make the cut off value conform to the sodium level of the blood stream but, if we vary the enzyme, the cut off value varies too It does not vary at as great a rate as might be expected For instance, if the enzyme is increased fivefold then the sodium concentration necessary for cut-off is increased ten or twenty milliequivalents In other words, the cut off is relatively insensitive to changes in enzyme concentration

Shannon What is your guide?

Binkley We have three variables in finding the cut off value for an ion One is substrate concentration, i e, how much glutamine or glutathione is present Two is the enzyme concentration and of course, the third one is the pH All these determine to some extent where the ion cut off comes

Shannon Forgetting about the three, in considering this in relation to the kidney, we can remove some of these because certainly pH is constant in the area where most of these ionic changes take place So that there are left then substrate concentration and enzyme concentration One would assume if this is so important to the architecture of the kidney as such that the enzyme concentration would be constant The reason I am bringing this up is I find the theory very attractive I am trying to find some point of reality to permit bringing it into the kidney and out of the test tube If one assumes that the enzyme concentration is constant, then one should

be able to take kidneys under different states with a high and low capacity to reabsorb ions and find very tight correlation between that function and the concentration of the substrate and the concentration of the glutamine contained in the system. So that the thesis is subject to experimental trial. I think if that point were tied down and if again it were demonstrated that the capacity of the system were such as to permit the parallel of a simple ion exchange resin of the type IRC-50 where one would get preferential handling of ions then we would have brought the system close to the kidney closer than it is now at the present time.

Binkley: We are working with intact animals now. We are very interested in a group of experiments in which we are using heavy nitrogen to trace the uptake in the amide groupings of protein and in the glutamine under different hormonal conditions to see if we cannot correlate these effects with the known effects of the hormones.

White: In hypophysectomized dogs where the PAH Tm is down to 20 percent of normal would it be possible or reasonable to try to determine whether the enzyme content of the kidney of such a dog is normal?

Binkley: We want very much to do that type of experiment. What we hope to do is use a dog with an explanted kidney so we can follow glutamine and glutathione in the renal and arterial blood. But I am not proposing that this mechanism is at the mercy of the blood supply. When the ammonia goes out in the urine so that the kidney loses ammonia then it must replenish the supply from the blood by removing glutamine from the blood.

Shannon: The thing which bothers me more than anything else is that the renal mechanisms possess high lability in terms of time. I am thinking of such phenomena as occur when one adds a minute quantity of the antidiuretic hormone in the course of two or three minutes the characteristics of the urine change completely, or if one adds very small amounts of phlorhizin as little as 12 mg per kg to a whole man one drastically upsets the reabsorptive mechanism for glucose. Again certain of the renal mechanisms possess a high degree of specificity as we have seen by indirect measurements. I have the feeling without being able to put my finger on it that this conception does not provide for the lability and the specificity that are so characteristic of renal activity.

During the past two years exchange resins have been built up into industrial chemical processes in a wide variety of situations. One can do almost anything with them. I am quite prepared to accept Dr Binkley's view because if you are limited to a simple

problem, regardless of what it is you can find an ion exchange resin to satisfy the particular need However when you use a resin to accomplish a highly specific end, then the conditions under which the process proceeds smoothly must be worked out in great detail with respect to the control of all variables Resins are used to extract penicillin from broth or streptomycin out of broth or crude solutions, and they are used in purification procedures of a wide variety in industrial procedures today, yet to obtain the control of variables to an extent where the specific operation has a high degree of efficiency requires extensive investigations Ion exchange resins are very susceptible in their action to minor changes in environment, such as in ion content, hydrogen ion concentration, and other variables They do have the potential of doing many specific jobs, but they do not possess the ability to perform many specific actions simultaneously

Binkley I would like to think of a tubule as a tube packed with ion exchange resin The distal tubule would be another column of practically the same thing arranged, if you wish, with a different architecture Then I am asking if the thin segment could not possibly act as some type of automatic stopper, so that flow is stopped or retarded until a certain ion concentration is reached in the proximal tubule

Grafflin In their extensive work upon fluorescence microscopy of the frog's kidney Ellinger and Hirt reported the jerky and rapid propulsion of the urine along the renal tubules Singer later confirmed this observation and spoke of a valve mechanism controlled *by the ciliated neck and intermediate segments* Dr Eisenberg and I likewise observed this rapid propulsion of the urine upon the ventral surface of the frog's kidney and it is exceedingly easy to demonstrate (Grafflin, A L and Eisenberg, M J, A micro fluoroscopic study of teleostean kidneys, *Anat Rec*, *59 449 (1934)*, this paper includes references to the publications of Ellinger and Hirt and of Singer) I don't know of any evidence for the occurrence of such a valve mechanism in mammals

Binkley I thought that such a valve might in some way change the whole character of more distal absorptive activity

Heller Are these ion exchange resins substances which show a greater affinity for potassium than sodium?

Binkley The carboxylic acid ones do not

Pitts I grant that you may doubt that the kidney is anion specific with respect to e' ' But for the sake of argument, let us ... extent than

could be explained by differences in ion mobility. Thus exchange resin concept could not account for anion specificity in absorption, could it?

Binkley: I don't know. I am surprised to find that the physiologists speak of absorption of sodium ions and water as separate processes. It would appear to me that it is much more reasonable to think of the ions as absorbed in the hydrated form so that each ion carries along with it a quota of water as water of hydration. In the first place the energy involved in the dehydration of an ion such as sodium would be considerable and thus an improbable mechanism. In the second place it is possible that some mechanism may be available for the modification of the hydration of an ion within wide limits and thus bring about changes in the amount of reabsorption of water.

Pitts: Is it not true that the energy produced in this reaction comes fundamentally from the hydrolysis of glutamine? You would dissociate sodium on the basis of this hydrolysis. Energy would be consumed in the resynthesis of glutamine, would it not?

Binkley: That is correct, at least initially. The hydrogen ion is a source of energy and can be used in the resynthesis of glutamine.

Pitts: How would you get around that if there were not?

Binkley: I can think of two sources. One is the production of hydrogen ion and the other is the production of glutamine. If enough hydrogen ions are produced then they can be exchanged for sodium.

Pitts: And by passing the glutamine?

Binkley: Just coming through. In such a case as sodium chloride the chloride must be following closely behind the sodium. But if on the other hand an anion cannot follow to the same extent that the chloride can follow, it may stay there and electroneutrality will be achieved by the movement of an hydrogen ion.

Shannon: It does not have to move at all.

Binkley: My guess is it tries to but cannot.

Shannon: If you consider a lattice of carboxyl groups sodiu can move across the lattice involved without disturbing electro neutrality or osmotic pressure.

Binkley: I think of the lattice as buffered so that at any one time there are not many acid groups.

Shannon: You knock one sodium ion off at one end of the lattice creating a capacity for a sodium ion at the other end. You don't create a situation wherein an anion has to move.

Binkley: Except to preserve neutrality if there is any accumula tion of positive ions on the other side.

Shannon You lose a hydrogen ion in the initial exchange so you don t disturb the system electrically

Binkley We are saying the same thing except that I am taking the other way around I am saying that the sodium ion is being moved and does create a charge The way to neutralize that charge is for hydrogen ion to go out

Pitts Do you interpret the effect of sulfanilamide on the acidity of the urine as being its effect on the glutaminase?

Binkley I am not too familiar with the details of the effects but do believe it can be explained on the basis of this mechanism

Wilhelmi If I understand you correctly there is a very intimate relationship between which ion happens to be on your resin and the actual enzymatic activity of the resin?

Binkley Yes

Wilhelmi How do you imagine that that is established?

Binkley The sodium ion and ammonium ion are competing with other ions this is one explanation I can offer

Shannon In relation to this sodium versus ammonium effect I would like to ask Doctor Pitts how long it takes his animals to come to equilibrium with the excretion of a maximal amount of ammonia

Pitts I cannot give you details of an experiment nor a very accurate time scale However I think there is a real time lag in the output of ammonia If you reduce the bicarbonate of the serum acutely from 25 to 10 millimols per liter by the intravenous infusion of hydrochloric acid over a period of two or three hours ammonia output will by no means have approached the maximum in that length of time If the same degree of acidosis is produced by giving hydrochloric acid by mouth over a period of several days ammonia excretion will reach much higher levels The greater ammonia output following oral administration of acid may in part be due to a time lag in ammonia output or it may be due to the greater depletion of stores of body base

Shannon I mean intravenous experiments where you bring it down abruptly and hold

Pitts Unfortunately we never carried those on past about four hours

Shannon Compared to chronic animals they did not have the same excretion rate of ammonia?

Pitts Not anywhere near

White That is not the experience of the Scandinavian worker Ryberg who found that ammonia production reached a plateau within 2 or 3 hours with no further increase in the next 24 or 48

hours (Ryberg, C On the formation of ammonia in the kidneys during acidosis *Acta physiol Scandinav* 15, 114 (1948))

Wesson Is it possible to predict from the properties of this system the effect of mercury on the excretion of these ions sodium potassium hydrogen and ammonium? What would be the effect on ammonium excretion for example?

Binkley I think I am logically obligated to say that it would reduce ammonia excretion

Leiter We have found that mercury has no effect on ammonia production in human subjects with or without heart failure

Mudge We found that mercurials had no apparent effect on ammonia excretion in the dog

Pitts We found no effect either

Wesson We found no significant change in one experiment

Pitts That is true also of the hydrogen ion concentration

Berliner We have not found mercurials to have any appreciable effect on either acidification or ammonia formation

Pitts That is what we found too

Binkley We have not tested whether the mercurial diuretics inhibit in the same fashion as mercuric chloride

Pitts I think the general concensus is that an organic mercurial diuretic acts by virtue of its liberation of ionic mercury that if one measures the diuretic response to bichloride of mercury it is essentially comparable to the organic mercurials in terms of mercury Furthermore there is not a great deal more damage done by bichloride than by the organic mercurials Small doses of bichloride of mercury are somewhat more nephrotoxic than the organic compounds but not of a different order of magnitude entirely

Taggart Certain of the observations which Dr Forster and I have made on isolated fish tubules oppose the idea that the organic mercurials are active only through the liberation of mercuric ions When tested as inhibitors of phenol red transport mercuric chloride and mercupurin were found to be approximately equal in activity on a molecular basis Such a finding would imply that the mercupurin was completely dissociated an unlikely possibility in view of the carbon mercury bond present in the organic mercurial In addition Hellerman and others have shown that one of the most effective inhibitors of the sulfhydryl enzymes is another organic mercurial p-chloromercuribenzoate

Leiter Would you be willing to grant that mercury acted on the proximal tubule rather than on the distal? This would explain the situation in regard to ammonia formation

BIOCHEMICAL ASPECTS OF RENAL TUBULAR TRANSPORT

JOHN V TAGGART

Department of Medicine College of Physicians and Surgeons
Columbia University

During recent years the development of micropuncture and clearance techniques has enabled the physiologist to describe various discrete renal processes in a very precise manner Through the use of these techniques abundant evidence has been obtained for a number of active secretory and reabsorptive mechanisms in the renal tubule The term active transport implies the movement of a solute across the tubular epithelium against a concentration gradient a process which necessarily entails the expenditure of energy derived from metabolic reactions within the cell Despite the recent progress which has been made in the fields of intermediary metabolism and renal physiology we know relatively little about the metabolic events which underlie active transport The only noteworthy exception is to be found in the case of glucose reabsorption The studies of Verzar and Laszt on intestine and of Lundsgaard and Kalckar on kidneys are too well known to need description here It is sufficient to say that a fairly strong case can be made for the stepwise phosphorylation and dephosphorylation of glucose in the cells of the proximal convoluted tubule The distribution of phosphatase in the luminal margins of the cells of this tubular segment as revealed by histochemical studies provides additional evidence in support of this concept

When our attention is directed to other transport systems such as those responsible for the secretion of diodrast phenol red or p aminohippurate no obvious mechanism is suggested by available biochemical information However it is reasonable to assume that the substance being transported undergoes a series of two or more reactions and that at least one of these involves the participation of an energy donating system Consequently certain importance attaches to the mechanisms by which metabolic energy is translated into osmotic work The utilization of phosphate bond energy in various biological syntheses in muscular contraction the propagation of the nerve impulse etc suggests its possible importance in

renal transport processes The implication of phosphate bond energy in selected transport mechanisms appeared to be a reasonable point of departure in the series of studies which I shall present this evening

It has long been recognized that at least certain of the aerobic oxidation reactions of the Krebs citric acid cycle are coupled with the generation of energy rich phosphate bonds Such aerobic phosphorylation can be readily demonstrated with the washed water insoluble particles of kidney homogenates The enzymes contained in the particles catalyze all of the reactions of the citric acid cycle and provide a final common oxidative pathway for the complete combustion of pyruvate fatty acids and certain amino acids This complex of enzymes has been given the name cyclophorase system (1) More recently it has been conclusively demonstrated that this enzyme complex is confined to the so called mitochondrial fraction obtained from tissue homogenates by differential centrifugation (2)

The coupling of phosphorylation with aerobic oxidation provides a mechanism whereby energy liberated by catabolic processes is made available for biosynthetic reactions and for the work of the cell The efficiency of this mechanism is quite remarkable During the oxidation of α ketoglutarate in the rabbit kidney cyclophorase system almost 3 molecules of inorganic phosphate disappear for each atom of oxygen consumed The inorganic phosphate has been incorporated into energy rich adenylpyrophosphates (ATP) The ATP generated from adenylic acid and inorganic phosphate can be measured by the addition of hexokinase and glucose to the system as each molecule of ATP is formed the hexokinase catalyzes the transfer of the terminal phosphate group onto the hexose to form hexose monophosphate a stable end product in this enzyme system By the use of this technique it is possible to approximate the P O ratio (moles of inorganic P esterified atom of oxygen) for each of the 5 oxidative reactions of the citric acid cycle The P/O ratio appears to be 3 for each of the reactions except that of succinate to fumarate in which it is 2 Thus at least 14 molecules of inorganic phosphate are raised to the energy rich level in the course of the five oxidative reactions which constitute the complete combustion of one molecule of pyruvate The maximal amount of energy which can be liberated by this series of reactions is theoretically sufficient to generate about 25 energy rich phosphate bonds Consequently the efficiency of the enzyme system *in vitro* is approximately 14/25 or almost 60 percent (3)

Of particular importance to the present discussion is the finding that 2 4-dinitrophenol (DNP), in extremely low concentrations, is capable of interrupting the coupling of oxidation and phosphorylation in such an enzyme system Essentially complete blocking of aerobic phosphorylation is obtained with M/10,000 DNP, while partial inhibition is observed with concentrations as low as M/100,000 Within this range of concentrations, the rate of oxygen consumption in the cell free system remains unchanged This should be contrasted with various other inhibitors of aerobic phosphorylation (e g, cyanide, azide arsenite or phlorhizin) which act by interfering with the electron transport system utilized in respiration We were first attracted to the study of DNP by the observations of Clowes and Krahl (4a, b) These investigators noted that DNP exerted a pronounced stimulatory effect on the respiration of fertilized sea urchin eggs, but at the same time arrested cell division Hotchkiss (4c) later made similar observations with suspensions of respiring staphylococci and yeast and noted in addition that the uptake of inorganic phosphate from the medium was blocked Thus, DNP produces an unusual combination of effects in intact cell systems, viz, respiratory stimulation and impairment of inorganic phosphate assimilation

A high degree of structural specificity underlies these effects of DNP The 2 and 4 mononitrophenols and even 2 amino-4 nitro phenol are completely inactive The phenolic group appears to be indispensable Various substitutions in the 6 position of 2 4-dinitrophenol have rather marked effects, alkyl and certain aromatic substituents enhance the activity of the parent compound, whereas a third nitro group (picric acid) abolishes activity In an attempt to uncover the role of phosphate bond energy in renal processes it seemed reasonable to examine the effects on tubular transport of a series of closely related nitrophenols, some of which had previously been shown to be active and some inactive in uncoupling aerobic phosphorylation The technique devised by Forster (5) by which the transport and accumulation of phenol red in isolated renal tubules of the flounder is observed microscopically, appeared to offer a very suitable test object for such studies Consequently, Dr Forster and I joined forces at the Mt Desert Island Biological Laboratory during the summer of 1948 and carried out the following studies (6)

Long segments of renal tubules are easily teased from the kidneys of freshly caught flounder When the tubules are suspended in a balanced isotonic saline solution containing dilute phenol red (1 mg

percent), a definitely detectable concentration of dye within the tubular lumina is usually apparent in 5 minutes The concentration of dye increases during the following 30 to 60 minutes to color intensities corresponding to 100 mg percent or higher The degree of phenol red accumulation was graded 0 to ++++, 0 indicating no accumulation, + a barely detectable concentration, and ++++ an intense red color corresponding to 100 mg percent or more Solutions of the various agents to be tested were prepared in the usual suspending medium and, when necessary, the pH was re-adjusted to that of the control Each agent was tested in concentrations ranging from M/10 000 to M/160 000 and comparison was made with an inhibitor-free control Only those experiments were considered satisfactory in which a ++++ concentration of dye was observed in the control within 30 to 60 minutes

The effects of the various nitrophenols on phenol red transport are summarized in Table I DNP inhibited transport completely at M/10,000, graded effects were obtained between M/20,000 and

COMPOUND TESTED	CONCENTRATION × 10^{-4} M				
	1 0	0 5	0 25	0 125	0.0625
	Activity of phenol red transport				
2 nitrophenol	++++	++++	++++		
4 nitrophenol	++++	++++	++++		
2-amino-4 nitrophenol	+++	++++	++++		
2,4-diaminophenol	+++	++++	++++		
2,4-dichlorophenol	++++	++++	++++		
2,4-dinitroanisole	+++	++++	++++		
2,4 6-trinitrophenol	o	o	o	+	++
2,4-dinitrophenol	o	+	++	+++	++++
2,4-dinitro-6-methylphenol	o	+	++	+++	+++
2,4-dinitro-6-phenylphenol	o	o	o	+	+
2,6-dinitro-4-chlorophenol	+	++	++	++	+++
2,6-dichloro-4 nitrophenol	+	++	+++	++++	++++

TABLE I Effect of various substituted phenols on phenol red transport in isolated renal tubules of the flounder (*Am J Physiol 161 167 1950*).

M/80,000, while no inhibition was apparent at M/160,000 This *gradation of effects was found to be remarkably reproducible It* is of interest that the degree of inhibition observed at each level of DNP corresponded closely to that previously obtained with the aerobic phosphorylation system of rabbit kidney In addition the inhibitory activities of the other nitrophenols of the series paralleled their effectiveness as inhibitors of aerobic phosphorylation with

one outstanding exception Trinitrophenol, or picric acid, which is inactive in the phosphorylation system, proved to be a very potent inhibitor of phenol red transport Picric acid may be recognized on chemical grounds to be an exceptional member of this series in another respect Substitution of a third nitro group in the 6 position greatly increases the dissociation of the phenol (pKa of 0 38 as compared with 4 0 for DNP) Therefore, it seemed possible that picrate blocked phenol red transport in some manner other than by interfering with aerobic phosphorylation It was suggested that picrate might differ from the other active nitrophenols by irreversibly inactivating some component of the transport system However, such is not the case When phenol red transport has been completely blocked by either DNP or picrate for as long as 20 minutes, transfer of the tissue to an inhibitor free solution results in a prompt uptake of dye

It then occurred to us that the action of picrate might be differentiated from that of the other active nitrophenols by an examination of the respiratory effects of the various agents These studies were performed in the Warburg apparatus with a crude mince of flounder kidney suspended in the usual saline medium Each agent was examined at a concentration which had previously been found to block completely phenol red transport, or in the case of the inactive agents at M/10,000 The addition of either phlorhizin, arsenite or sodium azide markedly depressed the rate of respiration On the other hand, DNP increased respiration to 75 percent above the control value Thus the characteristic stimulatory effect of DNP on fertilized sea urchin eggs, staphylococci and yeast was also obtained with fish kidney Examination of the 12 nitrophenols yielded the following results The 6 compounds inactive as inhibitors of either phosphorylation or phenol red transport had no significant effect on respiration the 5 compounds active in both systems stimulated respiration 39 to 75 percent Picrate, the previously noted exception had no effect on respiration One may reasonably conclude that the action of picrate on phenol red transport differs from that of the other active nitrophenols and is probably not primarily attributable to an interference with essential phosphorylation reactions

It is obviously impossible, on the basis of these preliminary observations, to assign a definite role to aerobic phosphorylation in the overall mechanism of tubular transport However, the generally excellent correlation between the results obtained with a mammalian kidney enzyme system and with the isolated renal tubules of the

flounder suggests that phosphate bond energy is utilized in the cellular transport of phenol red

Encouraged by these results, Dr Gilbert Mudge and I then examined the effects of DNP on various tubular transport mechanisms in the dog by the clearance technique (7) In each experiment three control period

intravenous dose of DNP

The study was continued

periods This dose of DNP always provokes some panting and a temperature elevation of about 1° C, but can be administered repeatedly at two week intervals over a period of months without causing weight loss or evidence of renal damage A typical experiment in which the maximal rate of PAH transfer (Tm_{PAH}) was measured is presented in Table II Administration of DNP resulted

TIME	C_F	V	p-AMINOHIPPURATE				RECTAL TEMP	PLASMA DNP
			P	UV	F	T		
min	ml/min		mg %	mg/min			°C	mM/L
0–24	53.2	0.64	46.0	35.3	24.5	10.8	38.5	
24–49	57.8	0.96	43.1	37.7	24.9	12.8	39.0	
49–63	56.5	0.77	44.8	37.3	25.3	12.0	39.0	
64–71	*2 4 Dinitrophenol 10 mg/kg i v*							
76–97	58.6	0.68	48.3	33.1	8.3	4.8	39.3	0.26
97–120	59.7	0.76	49.7	34.6	29.7	4.9	39.5	0.24
120–140	65.4	0.98	52.5	38.6	34.1	4.5	39.4	0.21

TABLE II Effect of a single intravenous injection of dinitrophenol on Tm_{PAH} Dog B wt 12 0 kg Glomerular filtration rate (C_F) was measured by creatinine clearance Tubular transport (T) represents the difference between the amount excreted (UV) and the amount filtered (F) PAH was infused at a rate of 36 mg/min (*Am J Physiol* 161 173 1950)

in a fairly prompt depression of the Tm to approximately 40 percent of the control value The plasma level of DNP as measured spectrophotometrically by a specific method fell from M/4000 to M/5000 during the period of observation Thus only partial impairment of PAH transport is apparent at plasma levels considerably above those required to block completely phenol red transport in the fish tubules However dialysis studies reveal that approximately 90 percent of the plasma DNP is protein bound Consequently the level of DNP in plasma water is actually about M/40 000 a concentration which exerts only partial effects on the two other systems previously mentioned Very similar results were obtained in a number of experiments Studies performed at low plasma levels of PAH such as are used in estimating renal plasma flow showed a similar degree of transport inhibition

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Encouraged by these results Dr Gilbert Mudge and I then examined the effects of DNP on various tubular transport mechanisms in the dog by the clearance technique (7) In each experi-

periods This dose of DNP always provokes some panting and a temperature elevation of about 1° C, but can be administered repeatedly at two week intervals over a period of months without causing weight loss or evidence of renal damage A typical experiment in which the maximal rate of PAH transfer (Tm_{PAH}) was measured is presented in Table II Administration of DNP resulted

TIME	C_F	V	P-AMINOHIPPURATE				RECTAL TEMP	PLASMA DNP
			P	UV	F	T		
min	ml/min		mg %	mg/min			°C	mM/L
0– 24	53 2	0 64	46 0	35 3	24 5	10 8	38 5	
24– 49	57 8	0 96	43 1	37 7	24 9	12 8	39 0	
49– 63	56 5	0 77	44 8	37 3	25 3	12 0	39 0	
64– 71	*2 4 Dinitrophenol 10 mg/kg i v*							
76– 97	58 6	0 68	48 3	33 1	28 3	4 8	39 3	0 26
97–120	59 7	0 76	49 7	34 6	29 7	4 9	39 5	0 24
120–140	65 4	0 98	57 5	38 6	34 1	4 5	39 4	0 21

TABLE II. Effect of a single intravenous injection of dinitrophenol on Tm_{PAH} Dog B wt 12 0 kg Glomerular filtration rate (C_F) was measured by creatinine clearance Tubular transport (T) represents the difference between the amount excreted (UV) and the amount filtered (F) PAH was infused at a rate of 36 mg/min (*Am J Physiol* 161 173 1950)

in a fairly prompt depression of the Tm to approximately 40 percent of the control value The plasma level of DNP, as measured spectrophotometrically by a specific method fell from M/4000 to M/5000 during the period of observation Thus only partial impairment of PAH transport is apparent at plasma levels considerably above those required to block completely phenol red transport in the fish tubules However dialysis studies reveal that approximately 90 percent of the plasma DNP is protein bound Consequently, the level of DNP in plasma water is actually about M/40 000 a concentration which exerts only partial effects on the two other systems previously mentioned Very similar results were obtained in a number of experiments Studies performed at low plasma levels of PAH, such as are used in estimating renal plasma flow showed a similar degree of transport inhibition

one outstanding exception Trinitrophenol or picric acid which is inactive in the phosphorylation system proved to be a very potent inhibitor of phenol red transport *Picric acid may be recognized on* chemical grounds to be an exceptional member of this series in another respect Substitution of a third nitro group in the 6 position greatly increases the dissociation of the phenol (pKa of 0 38 as compared with 4 0 for DNP) Therefore it seemed possible that picrate blocked phenol red transport in some manner other than by interfering with aerobic phosphorylation It was suggested that picrate might differ from the other active nitrophenols by irre versibly inactivating some component of the transport system However such is not the case When phenol red transport has been completely blocked by either DNP or picrate for as long as 20 minutes transfer of the tissue to an inhibitor free solution results in a prompt uptake of dye

It then occurred to us that the action of picrate might be dif ferentiated from that of the other active nitrophenols by an examina tion of the respiratory effects of the various agents These studies *were performed in* the Warburg apparatus with a crude mince of flounder kidney suspended in the usual saline medium Each agent was examined at a concentration which had previously been found to block completely phenol red transport or in the case of the inactive agents at M/10 000 The addition of either phlorhizin arsenite or sodium azide markedly depressed the rate of respiration On the other hand DNP increased respiration to 75 percent above the control value Thus the characteristic stimulatory effect of DNP on fertilized sea urchin eggs staphylococci and yeast was also obtained with fish kidney Examination of the 12 nitrophenols *yielded the following results The 6 compounds inactive as* inhibitors of either phosphorylation or phenol red transport had no significant effect on respiration the 5 compounds active in both systems stimulated respiration 39 to 75 percent Picrate the previously noted exception had no effect on respiration One may reasonably con clude that the action of picrate on phenol red transport differs from that of the other active nitrophenols and is probably not primarily attributable to an interference with essential phosphoryla tion reactions

It is obviously impossible on the basis of these preliminary observations to assign a definite role to aerobic phosphorylation in the overall mechanism of tubular transport However the generally excellent correlation between the results obtained with a mammalian kidney enzyme system and with the isolated renal tubules of the

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			P	UV	F	T		
min.	ml/min		mg %	mg/min			°C	mM/L
0– 24	53 2	0 64	46 0	35 3	24 5	10 8	38 5	
14– 49	57 8	0 96	43 1	37 7	24 9	12 8	39 0	
19– 63	56 5	0 77	44 8	37 3	5 3	12 0	39 0	
54– 71	*2,4 Dinitrophenol 10 mg/kg i v*							
76– 97	58 6	0 68	48 3	33 1	28 3	4 8	39 3	0 26
97–120	59 7	0 76	49 7	34 6	29 7	4 9	39 5	0 24
20–140	65 4	0 98	52 5	38 6	34 1	4 5	39 4	0 21

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Apparently a number of tubular excretory processes can be depressed by DNP administration, since very similar results were obtained with both diodrast and phenol red Studies on certain tubular reabsorptive mechanisms yielded very different and somewhat surprising results Glucose Tm remained unaltered after the usual dose of DNP, nor was there any appreciable effect on the tubular reabsorption of glycine, inorganic phosphate, sodium or potassium We are unable to explain adequately why the inhibitory activity of DNP appears to be limited to tubular excretory mechanisms Certainly one should expect that the impairment of aerobic phosphorylation would result in a significant reduction of the glucose transport capacity However, it should be emphasized that doses of DNP tolerated by the intact animal probably produce only a partial depression of aerobic phosphorylation We may be dealing simply with a quantitative rather than a qualitative difference The energy required to support either type of transport at a maximal rate remains unknown There is the additional possibility that DNP is selectively localized at the peritubular margins of the renal cells and, consequently exerts its most striking effects on the transport processes which are initiated at that site In this connection I can add only that limited analyses of renal cortex have failed to reveal any significant concentration of the drug Finally, it is quite possible that phosphate bond energy is utilized in quite a different manner in PAH and diodrast transport as compared with that of glucose Since there is little reason for believing that either of the former compounds undergoes phosphorylation during transport, it appears much more likely that it is one of the cellular components of the secretory mechanism which must be 'primed' through interaction with an energy rich phosphate Certain of the studies which follow support this idea

Throughout the course of our studies we felt the need for relatively simple *in vitro* techniques to facilitate biochemical studies on transport, techniques which would be more readily available in our city [illegible]
mammali[illegible]
early in [illegible]
the accumulation of glutamate by brain slices (8) [illegible]
members of our group Dr Richard Cross, soon found that this technique could be readily adapted to serve our purpose (9)

Thin slices (0 3 mm) of rabbit kidney cortex are prepared as for respiration studies Approximately 300 mg of tissues are suspended in a Warburg vessel in 2 7 ml of a suitable isotonic saline medium

containing dilute PAH The gas space is filled with oxygen and the vessel is shaken in a 25° water bath for the desired time Oxygen consumption is measured and recorded as a QO_2 (cmm of O per milligram of wet wt of tissue) At the termination of the experiment the slices are quickly removed blotted on filter paper weighed and extracted with 3% trichloracetic acid a filtrate of the medium is also prepared for estimation of PAH Figure 24 shows the results obtained with varying periods of time It is evident that the concentration of PAH in the kidney slices rises rapidly at the expense of that in the medium To express the gradient established we have employed simply the concentration ratio slice/medium (S/M) If

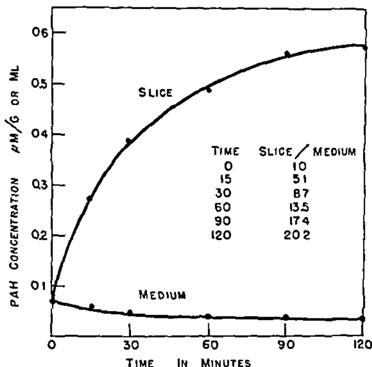


FIGURE 24

this group of inhibitors Carinamide, examined because of its known capacity to inhibit a number of *tubular excretory processes*, *was* effective at M/5000 Two representative substances, diodrast and penicillin, which are excreted in part by the renal tubule, blocked PAH accumulation at concentrations of M/1000 and M/250 respectively Inasmuch as the last three compounds have no appreciable effect on respiration, it seems probable that they compete with PAH for the transport system Incidentally, I was interested in Dr Binkley's earlier *statement that penicillin is much more effective* than carinamide as an inhibitor of glutaminase We have found quite the reverse, carinamide is approximately 20 times as active as penicillin G

The slice technique, therefore, has yielded results which are in good agreement with those obtained by clearance methods Another series of observations provides some evidence of the usefulness of this *experimental procedure Since acetate* had been shown to stimulate PAH accumulation in a striking and consistent manner, Dr Mudge examined the effect of acetate infusions on Tm_{PAH} in the dog (10) Table IV presents a typical experiment in which the

TIME	C_F	V	P-AMINOHIPPURATE				Tm / AVERAGE CONTROL
			P	UV	$C_F \times P$	Tm	
min	ml/min		mg %	mg/min			%
−46	NaCl infusion started, 140 mEq/l at 10 ml/min i v						
−44	3 gm creatinine and 3 gm PAH in 75 ml water i v						
−39	Sustaining infusion started 7 5 mg creatinine and 23 mg PAH per ml in isotonic NaCl at 2 ml /min						
0–10	69 3	5 2	47 1	46 1	32 7	13 4	96
10–21	70 2	5 5	44 0	44 3	30 9	13 4	96
21–31	76 5	7 9	41 1	46 4	31 4	15 0	108
31	NaCl infusion replaced with Na acetate infusion 140 mEq/l at 10 ml/minute						
31–41	72 5	8 2	40 5	46 4	29 4	17 0	122
41–55	71 2	6 0	36 5	45 2	26 0	19 2	138
55–75	71 3	4 7	33 8	46 4	24 1	22 3	161
75–95	67 9	6 3	34 1	46 3	23 1	23 2	167

TABLE IV Effect of sodium acetate infusion on Tm_{PAH} Dog S wt 17 0 kg Experiment demonstrates an increase in Tm_{PAH} without significant change in the filtration rate (C_F) or urine volume (V) erum bicarbonate rose from 22 1 mEq/l in period 3 to 28 2 in period 7 *(Am J Physiol, 161, 191, 1950)*

infusion of 82 μM of sodium acetate per kg per min resulted in elevation of the Tm_{PAH} to 67 percent above the average control values The increase in Tm occurred independently of any significant change in either the glomerular filtration rate or urine volume In 9 other experiments in which the rate of acetate infusion ranged

from 66 to 210 μM/kg /min Tm_{PAH} was increased 44 to 85 per cent Lactate infusions produced a similar increase On the other hand succinate or fumarate administered in the same manner de pressed the Tm 20 to 47 percent below the control values confirm ing the results with the slice technique Studies of the plasma elec trolytes during administration of the various substrates revealed that acetate is metabolized at a very rapid rate whereas significant amounts of lactate succinate and fumarate accumlate in the plasma In view of the possibility that acetate might be effective because of its alkalinizing effect additional studies were performed with sodium bicarbonate infusions In one of these there was a slight depression of Tm_{PAH} in two others a slight stimulation It was quite clear from these experiments that bicarbonate administration did not produce effects quantitatively similar to those of acetate despite a comparable degree of alkalosis It is also improbable that the increase in Tm_{PAH} induced by acetate is due to the opening up of previously inactive nephrons since acetate had no demon strable effect on the reabsorptive Tm of glucose

These last observations serve two functions First they establish the usefulness of the slice technique in exploratory studies on the biochemical mechanisms involved in renal transport Second they focus our attention more sharply on the possible importance of acetate as a rate limiting cellular component of the PAH transport system The very fact that there are maximal rates of tubular transport implies that certain cellular components of the system are available in limited quantities Acetate is not an unlikely candi date for such a role Although isotope dilution studies have shown that the daily turnover of acetate is relatively tremendous its concentration in cells and body fluids at any given time is vanish ingly small In addition the biochemist has become increasingly aware in recent years of the multitude of biological reactions in which acetate participates The analysis of certain of these reactions such as the acetylation of foreign amines or choline and the forma tion of acetoacetate or citrate reveals that acetate as such is not involved but a more reactive C compound derived from acetate or its precursors through interaction with energy rich phosphates Though the nature of this active acetate is not yet known it is quite certainly not acetyl phosphate in mammalian systems Nor can we describe the manner in which acetate enters into the re actions of PAH transport All available evidence points away from acetylation of the p amino group as being an important reaction If active acetate does play an important role in this

it must be regarded as a potential donor of either acetyl or phos phate groups

You must be quite aware at this point that the studies presented do not provide a very complete description of any one of the active transport mechanisms We are encouraged however by the fact that there has been such good agreement between the observations obtained with a cell free enzyme system with isolated tubules and kidney slices and with the intact animal By approaching the problems of transport at each of these levels of cellular organization we feel that it is reasonable to anticipate further progress in un covering certain of the biochemical reactions involved

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(4) a [illegible] all meta

b Krahl M E and Clowes G H A Studies on cell meta bolism and cell division II Stimulation of cellular oxidation and reversible inhibition of cell divis on by dihalo and tri halophenols *J Gen Physiol* 20 *173 (1936)*

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DISCUSSION

Pitts There is a point upon which I would like your reaction In connection with the general problem of competitive inhibition is inhibition on the basis of competition for some cell component present in limited amounts or is there some limitation of energy available to the cell? Do your results have any direct bearing on this question?

Taggart The inhibitors which we have studied thus far would appear to fall into three categories First there are those which interfere with one or another step in electron transport phlorhizin arsenite azide and cyanide are representative By restricting the oxidative activities of the cell they limit the amount of energy which can be liberated Second the active nitrophenols permit oxidative reactions to proceed at an accelerated pace but they paralyze the mechanism whereby the energy liberated is diverted into useful work Lastly there are such compounds as diodrast PAH phenol red and penicillin which appear to compete with one another for some cellular component of the transport system Since we know so little about the energy requirements of the various transport systems it is indeed difficult to say whether the available energy ever represents the limiting factor

Heller By what mechanism is dinitrophenol excreted by the kidney?

Taggart We have done a limited number of clearance studies with dinitrophenol It is cleared at a rate of considerably less than 1 cc per minute Taking into account the protein binding of plasma DNP the small amount which is filtered must be fairly completely reabsorbed There is little reason to believe that DNP competes with PAH for the excretory mechanism

Warren In your studies with dinitrophenols did you find increased oxygen consumption?

Taggart We have never measured oxygen consumption in the intact animal but studies by Tainter have shown that it is appreciably increased in dogs following the administration of doses such as we have employed We have also observed as did Tainter that

venous blood shows a lower oxygen content after DNP administration Incidentally, in one experiment in which the right renal vein was catheterized the renal venous blood also appeared less saturated than usual In that experiment, DNP lowered the clearance of PAH at low plasma levels approximately 50 percent, but the renal plasma flow calculated by the Fick principle had decreased only 10 percent In short, the effect of DNP was primarily on the extraction of PAH rather than renal hemodynamics

Leiter Do you know whether under the conditions of the renal clearances there is actually an increase in oxygen consumption?

Taggart I do not know of any data on renal oxygen consumption during active tubular transport of a foreign substance

Binkley I find a little bit of concern over this proposed mechanism of action of dinitrophenol I was wondering, for instance, if anyone has tested the simple idea that there might be an activation of phosphatases

Taggart I believe that such studies have been done in Cori's laboratory It is my understanding that no effect on the generation of ATP was observed during anaerobic glycolysis In the present studies we would be primarily concerned with the possible activation of adenosinetriphosphatase by DNP We have found that DNP does not have this effect

Binkley We find that the small acids are activators of glutaminase We find that the larger acids are inhibitors and most, if not all, dicarboxylic acids are inhibitors I don't know what significance may be attached to these findings

Taggart We have been hard pressed to find an adequate explanation for the inhibitory actions of so many substrates, especially those of the citric acid cycle All of the latter stimulate respiration and their oxidation should provide additional available energy for transport We have considered the possibility that an acceleration of the cycle depletes the cells of available acetyl groups through the formation of tricarboxylic acids, but that is not a very attractive idea Moreover, we find it impossible to reverse the effect of α ketoglutarate by the addition of an excess of acetate Of course, the rate at which acetate diffuses into the cell may be a limiting factor in this situation

Selkurt Since one can increase PAH Tm with acetate or depress it with dinitrophenol, have you ever tried using both in a single experiment?

Taggart Acetate cannot completely reverse the effect of dinitrophenol If one first increases PAH transport with acetate and then

administers dinitrophenol the final rate of transport is considerably below the control values

Pitts Suppose you were to give p aminohippurate and then depress that Tm with penicillin and then raise it again with acetate what have you done with a concept of Tm and the factor which causes limitation?

Taggart Of course we too wonder just was Tm means In the past we have regarded any particular Tm as a sort of fingerprint for each individual Is it not probable that the relative constancy of a Tm as usually measured simply reflects a fairly constant set of experimental conditions?

Pitts It is now surprising to me that Tm should be in an animal or man on successive measurements as nearly constant as it is

Mudge We measured Tm in one dog 12 times in 8 months and were impressed by the fact that the value was not very constant Tm varied from 9 to about 16

Pitts We have a series of experiments in which we have been testing in dogs the toxicity of two mercurial compounds One of our measures has been p aminohippurate Tm I dont think Tm varied more than plus or minus 10 percent in a large series of measurements

Forster Is that the case with glucose Dr Shannon?

Shannon That was our experience

Taggart It has occurred to us that the different values of Tm obtained from time to time may reflect spontaneous variations in the concentration of acetate in the cells

Pitts Would you go so far as to say although it is very low it is maintained under usual conditions at a fairly stable concentration?

Taggart I would think so

Pitts Constancy of Tm would be a function of the constancy of whatever cellular mechanism maintained cellular acetate

Wilhelmi There is another possibility It may fluctuate with the nutritional state of the animal If one considers the possibility that the so called active acetate is a rather complex substance then it might not be so much the concentration of acetate in the tissue at any particular time as the rate of production of acetate together with the quantity of material that can form the active acetate complex so that the two things together might easily determine for any given instance — if this mechanism is specific for the p aminohippurate transport — just what the Tm would be

Taggart That is exactly what we would like to believe — that the

Berliner In that connection, it is of some interest that urate reabsorption is depressed by diodrast but not by hippurate (Talbott, J H Gout *Oxford University Press*, New York (1943))

Wilhelmi With respect to the point that Dr Shannon made a while back that the mechanism of transport might in some instances be the limiting factor in transport, there are some experiments done long ago by Auchinachie, Macleod and Magee (Auchinachie, D W, Macleod, J J R, and Magee, H E Studies on diffusion through surviving isolated intestine, *J Physiol* 69, 185 (1930)) on the absorption of glucose from isolated loops of rabbit intestine They noticed that at the concentrations of glucose which in the active aerated intestine led to maximal absorption, the absorption of sugar after the intestine had been killed with hot water was in fact much greater So that in that instance too the transport mechanism seemed in fact to be a limiting factor in the regular departure of glucose from the lumen of the rabbit loop to the outside fluid There might be another instance comparable to that which Dr Shannon mentioned, in which the mode of the movement through the cell, whatever mechanism it is, provides an obligatory pathway which can be filled only to a certain extent In such an instance the rate of energy supply would not necessarily be a limiting factor in the transport A comparable instance of competition arises in the sense that if one administers glucose and galactose simultaneously the total absorption is not summated but each sugar is absorbed at a rate rather less than that at which the sugars individually would pass into the blood stream

Forster Would you say, Dr Taggart, that the dependence upon oxygen as an energy source is pretty well established for kidney function in contrast, for example, to nerve action or muscle action?

Taggart There is certainly little doubt in my mind that active tubular transport depends primarily on aerobic processes for its energy supply If anaerobic glycolysis could provide the energy needed dinitrophenol should not have such a pronounced effect on transport In addition, we have already noted that the maintenance of relatively high oxygen tension is required for transport in both isolated fish tubules and rabbit kidney slices One wonders whether this is not related to the fact that the kidney is perfused throughout its vascular bed by blood of relatively high oxygen content

Pitts As a renal physiologist I have always operated on an assumption which I took finally to be almost law, namely, if a substance is reabsorbed, it is not secreted, if a substance is secreted,

it is not reabsorbed In reality this assumption underlies all measurements of either secretory or absorptive capacity Reasoning teleologically, *if the body considers it sufficiently worth while to get* rid of a substance to develop a secretory mechanism to do so why should it develop a mechanism to reabsorb that substance Not too long ago Dr Berliner and his colleagues and Dr Mudge and his colleagues began tapping on what we thought were firm foundations for renal physiology and found them not so solid Just how much of those foundations have been riddled and wormholed I don't know Dr Berliner is to introduce the topic of tubular secretion of potassium and I hope will point out some of the discrepancies in our basic assumptions

THE TUBULAR SECRETION OF POTASSIUM AND ACID

ROBERT BERLINER

Department of Medicine College of Physicians and Surgeons, Columbia University

I have been asked to introduce a discussion of the excretion of acid and potassium by the kidney The matter of the secretion of acid is one about which Dr Pitts is much better qualified to speak and anything I may say will be more or less a summary of his work with which you are all familiar I will, for the most part, confine myself to the excretion of potassium with which we have worked I should like to say at this point that the material for this presentation represents the joint efforts of Drs Thomas J Kennedy, Jr, Dr James G Hilton, and myself (2)

Although the excretion of acid and the excretion of potassium may not be particularly closely related, certain similarities may warrant their being considered jointly The first similarity is concerned not so much with the mechanisms involved but with our thinking about them For a long time it was assumed, and not unreasonably, that the excretion of both was regulated by filtration and reabsorption In both cases it had been shown that the amount excreted did not exceed the amount filtered under ordinary circumstances The amount of acid excreted could be explained by the filtration of buffers and the reabsorption of the more basic com ponents — bicarbonate and dibasic phosphate, the average daily excretion of potassium is only about 10% of the amount filtered, and therefore easily accounted for by filtration reabsorption However in both cases when conditions were modified so as to produce marked increases in excretion, it became apparent that the amount filtered was not sufficient to yield the amount excreted You are all familiar with Dr Pitts' work which showed that the reabsorption of bicarbonate and phosphate was inadequate to explain the titratable acid in the urine under properly chosen experimental conditions and that hydrogen ions must therefore be secreted We will for the present, omit consideration of the subsequent proposal of Menaker (7) that reabsorption of carbonate might account for the acidification of the urine since this would make available as hydrogen ion, the hydrogen of bicarbonate I personally don't think this is a very likely mechanism but perhaps someone would like to bring

it up in the subsequent discussion Turning to potassium we find a similar situation The fact that potassium might be secreted was suggested by isolated observations of McCance (6) and of Keith Osterberg and King (4) which were more or less completely over looked by workers in renal physiology including ourselves McCance observed a potassium clearance of 11 with an inulin clearance of 9 in a patient with severe alkalosis Some question as to the validity

little data but stated that in a normal subject who developed diarrhea and a drop in filtration rate after an oral dose of KCl they observed a potassium clearance 32% greater than the inulin clear ance Our conclusion that there is a secretory mechanism for potas sium was based on experiments similar to that shown in Table V

Time min.	Urine Flow ml/min.	Creatinine Clearance ml/min.	Inulin Clearance ml/min.	Plasma Potassium meq/L.	Filtered Potassium μeq/min.	Excreted Potassium μeq/min.	Ratio Excr./Filt
0	Start infusion of creatinine inulin and thiosulfate in 0.3 N KCl at 1ml/min						
68 90	385	56	56	52	279	350	1 25
112	545	63	64	51	313	454	1 45
128	589	71	71	50	340	513	1 50
146	545	72	70	53	356	525	1 47
150 167	Infusion of 75 mM of sodium bicarbonate in 500 ml. of water						
170 184	1118	77	75	43	3 8	476	150
199	787	80	78	45	346	510	48
214	640	76	76	45	328	484	148
229	480	82	80	45	354	487	137

TABLE V

This experiment was performed on a normal female dog which had received by mouth 5 gms of KCl twice a day for one week We'll consider the significance of the oral KCl later The dog received an infusion of *creatinine* inulin and thiosulfate in KCl throughout the study potassium being infused at a rate of 0 5 mEq per min You can

see that the potassium was excreted at just about the rate it was infused, which is some 40% or more greater than the product of plasma potassium and creatinine clearance The dose of bicarbonate was given to test a suggestion that creatinine might be reabsorbed from alkaline urines The comparison with inulin does not suggest that this was the case In fact we have, during a number of studies of potassium excretion in dogs, measured the filtration rate with two or more substances always including creatinine with uniformly good checks It is clear from this experiment and similar experiments in 13 other dogs that there must be a tubular mechanism for adding potassium to the urine The question now arises as to the role of secretion in the normal excretion of potassium Are we to assume that secretion plays a part only when the excreted potassium *exceeds the filtered? Certainly this is the only time at which it can* be unequivocally demonstrated Here the situation again resembles that for acidification of the urine — under all normal circumstances *bicarbonate reabsorption is adequate to explain the excreted titra*table acid, but it is not safe to assume that there is no hydrogen ion secreted unless it can be unequivocally demonstrated Dr Pitts has *in fact, offered evidence for acid secretion even* when the urine is highly alkaline The same might be true for potassium secretion There is at present, no way of arriving at a certain conclusion All we can measure is the net effect of opposing reabsorption and secretion, so that quantitative evaluation of either process alone is not feasible

Let us turn to a consideration of some of the factors which may determine the rate at which potassium is excreted You may recall that in Table V shown a short time ago the plasma potassium was only very slightly elevated in the first group of periods and close to the average normal value after the dose of bicarbonate, yet the excretion of potassium was at a very high level — say some ten times as high as if this dog had, with the same plasma potassium, been receiving sodium chloride rather than potassium *chloride*

Shannon Do you have the sodium concentrations, Dr Berliner?

Berliner I did not have room in this Table for all the data but *the plasma sodiums were well within the normal* range which, in the dog is a little higher than in man, 140 to 145 mEq per liter

This will illustrate, then, one of the most striking features of *potassium excretion — a very marked dissociation of the rate of* excretion from the plasma concentration, or perhaps more important from the standpoint of our usual thinking in renal physiology, a dissociation of the rate of excretion from the filtered load This, and

an additional point, may be further illustrated by the data plotted in Figure 25 This represents a number of experiments in the same dog in each of which potassium was infused, but in combination with different anions Before every experiment the dog had received

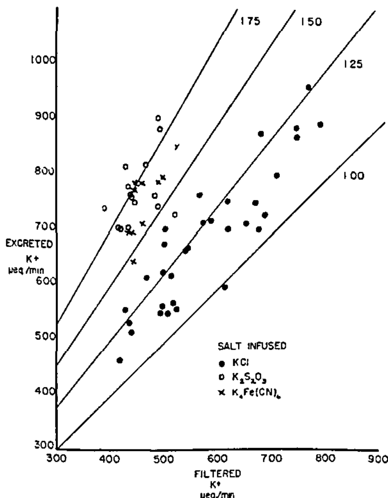


FIGURE 25

oral KCl for at least one week You can see that at any filtered load there may be a wide range of amounts excreted A part of the variation, but by no means all, may be explained by the differences

obtained with different anions The highest ratios of excreted to filtered – and the greatest absolute excess of excreted over filtered – are regularly obtained by the infusion of *potassium thiosulfate, the* lowest with the chloride, while ferrocyanide generally *gives intermediate* values although the difference between *thiosulfate and* ferrocyanide is not so well shown here The explanation of *this effect of the anion lies, we believe, in the demand they create for* cation excretion by the obligatory excretion of anion Chloride can be almost completely reabsorbed by the tubules so that the extent to which it holds cation in the tubular fluid is subject to variation Ferrocyanide, on the other hand, is not reabsorbed so that an amount of cation at least equal to the filtered and actually equal to the amount of ferrocyanide infused must be excreted Thiosulfate *is excreted without reabsorption, but urines obtained during the* infusion of thiosulfate contain about as much sulfate as thiosulfate, and the required cation excretion is greater than the amount infused We have found incidentally, that the infusion of p aminohippurate facilitates the demonstration of K secretion in man We may say, then, that the rate of excretion of potassium is at least partly determined by the rate of excretion of anion

Another factor which accounts for part of the *variability in the* relationship of excreted to filtered was alluded to when it was mentioned that the experiments were preceded by a week of oral KCl This is the factor of tolerance, the results of which are illustrated by Figure 26 This represents two exactly similar experiments on the same dog The first of these was the initial experiment on the dog, the second was preceded by the oral administration of 4 gms of *KCl twice a day for two weeks In each KCl was infused at a rate* of 500 μEq per minute, one hour allowed for equilibration and eight 20 minute clearance periods obtained In the first experiment, potassium excretion lags, falling well below the amount infused during the greater part of the experiment Consequently the plasma potassium becomes moderately elevated and the amount excreted remains well below the amount filtered When tolerance has been developed, the rate of excretion has, *within the hour's equilibration*, become equal to the rate of infusion The plasma potassium is considerably lower than in the previous study and the excreted exceeds the filtered throughout

Bradley How long does it take for tolerance to develop?

Berliner I don't know Five days is plenty, but we have not really explored it to find out We were at the time not particularly inter-

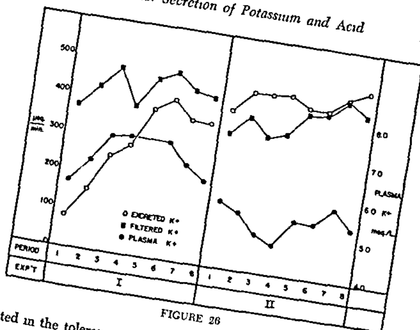


FIGURE 26

ested in the tolerance except so far as it got us where we wanted to go

The nature of this tolerance has not been clarified It is not attributable to a continuously high potassium excretion before a dose is administered The rate of potassium excretion in the tolerant animal than in the dog which has previously received no potassium is not greater We have tried, without success, to reproduce the tolerance by the administration of desoxycorticosterone Given in large doses for a short period before an experiment the DOCA was without demonstrable effect Given over longer periods such potassium depletion was produced that the administered potassium was almost quantitatively soaked up and very little was excreted When the K excreted in excess of intake in response to DOCA was readministered, the results were inconclusive During the infusion of potassium salts, large doses of desoxycorticosterone acetate in oil intramuscularly, or in propylene glycol intravenously, or large doses of desoxycorticosterone glucoside intravenously had no effect on potassium excretion These findings are in accord with those of Thatcher and Radike (12) who first described the phenomenon of tolerance to K in rats and found that they were unable to reproduce the tolerance with DOCA While there remains no question of the fact that adrenal steroids affect potassium excretion

the effect is not clear, nor is there any clear relationship between the adrenals and the development of tolerance

As to the nature of the factors which determine potassium excretion and their quantitative relationships we can draw no final conclusions It seems likely that potassium excretion is determined at least partly by intracellular potassium concentration and possibly by the renal cellular potassium concentration Determination of these concentrations does not at present seem feasible I believe it is Dr Mudge's interpretation, that the evident secretion of potassium in their experiments with forced osmotic diuresis with urea, is attributable to the increased intracellular potassium concentration attendant upon cellular dehydration

Leaving these most important questions more or less unsettled, another aspect of the mechanism of excretion warrants consideration You will recall that Dr Pitts suggested that the acidification of the urine is accomplished by an exchange of hydrogen ions for sodium ions The reciprocal relationship between sodium and potassium excretion under certain circumstances suggested that a similar process might be involved in potassium secretion If a urine could be obtained which contained essentially one anion which could not be secreted and if this urine contained potassium in excess of that filtered, then the potassium would have to be present in place of

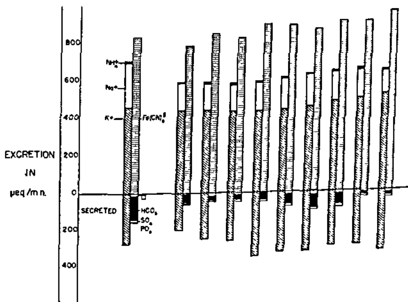


FIGURE 27

some filtered cation It seemed desirable to use an anion which is excreted at the level of glomerular filtration since there would then be some assurance that this anion was not secreted or reabsorbed For this purpose ferrocyanide was selected Figure 27 will illustrate one of a number of experiments in which potassium ferrocyanide was infused After an hour and a half a series of 20 min clearance periods were obtained The excretion of K^+ Na^+ NH_4^+ ferrocyanide, Cl^- bicarbonate SO_4^- and phosphate were measured as well as the creatinine clearance and plasma potassium In plotting this figure the excreted potassium has been divided so that the amount filtered is above horizontal line the minimum amount secreted below On the anion side the ferrocyanide is plotted above the line since there is fair evidence that it cannot be secreted The other anions are plotted below although at least for sulfate and phosphate there is fairly good evidence that they are likewise not secreted It is worthy of note that the sum of the measured anions checks fairly closely with the total of measured cations the anions being in slight excess possibly partly accounted for by Ca and Mg which were not measured In any case it seems unlikely that there is any large amount of some anion which has been overlooked You can see that throughout the experiment the secreted potassium s far in excess of the total of anions which for present purposes e may concede might have been secreted with it In other words any anion has been secreted with the potassium it is not appearing the fully elaborated urine Three possible mechanisms which might account for these findings are presented in Figure 28 Those are analogous to those suggested for the secretion of acid The first represents secretion of the potassium salt of some acid such as hydrochloric with subsequent reabsorption of the anion as the sodium salt If this were the case one might expect to find a consistent increase in the excretion of the anion The second represents secretion of the bicarbonate with subsequent reabsorption of sodium bicarbonate by the mechanism suggested by Dr Pitts Were this mechanism operative one might anticipate a consistent increase in bicarbonate excretion The last represents a direct exchange of potassium ions for sodium ions

It has been well known for a long time (Loeb et al (5)) that increasing excretion of potassium is usually associated with a tendency for the urine to become alkaline An investigation of this phenomenon seemed indicated for the light it might throw on the mechanism for the secretion of potassium An attempt was made to establish conditions in which the results obtained would be relat

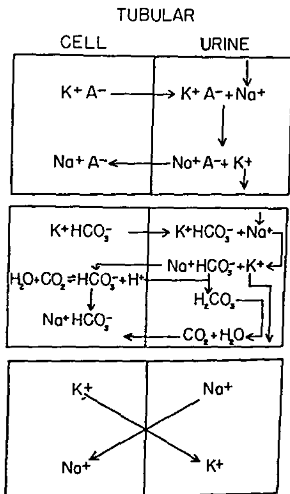


FIGURE 28

to the administration of potassium and independent of the anion administered with it To accomplish this experiments were done which started with the infusion of the sodium salt of one of several anions Similar results were obtained with the ferrocyanide the thiosulfate and the phosphate The experiment illustrated in Figure 29 was done with ferrocyanide Sodium ferrocyanide was infused at a rate of 1200 microequivalents a minute After 90 minutes to reach a more or less steady state, clearance studies were begun and the infusion was arranged so that the sodium was progressively replaced by potassium at equivalent concentration keeping the

ferrocyanide constant The change from sodium to potassium was started at the end of the first 20 minute clearance period and was 90% complete two hours later Soon after the infusion of potassium was started there was a sharp rise in chloride excretion and in bicarbonate excretion as indicated here by the pH These changes were not associated with changes in the amount of these anions filtered since the chloride filtered remained constant and the bicarbonate fell progressively They were therefore in some way related to the change in potassium excretion The increase in anion excretion however was maximal before the excretion of potassium had reached the amount filtered The excreted potassium exceeded that filtered by a small margin in the sixth period Note that the sodium excretion remained at its initial high level until the sixth period and that its fall is associated with stabilization of the excretion of chloride and bicarbonate

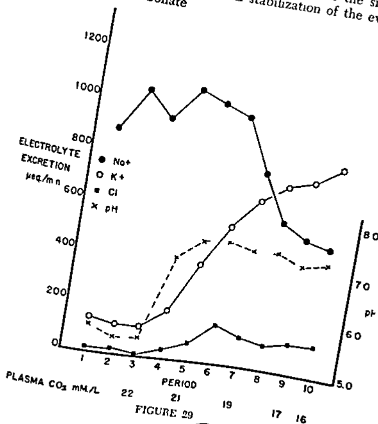


FIGURE 29

No conclusive interpretation of these experiments can be made, but we can narrow it down to two alternatives We may start by *assuming that* reabsorption and secretion cannot proceed simultaneously in the same segment of the tubule, and from what is known of the site of various tubular processes (Richards (11), *Walker, et al (13)*) we may place the reabsorptive process as most probably in the proximal tubule The secretory process must then be distal Distal secretion of the potassium salts of chloride *and* bicarbonate seems unlikely especially in view of experiments like that shown in the previous Figure since a still more distal site for reabsorbing the sodium salts would be required The observed findings could be explained by a) a decrease in the proximal reabsorption of potassium and the associated anions b) a diversion of distal reabsorption from reabsorption of sodium with chloride and bicarbonate to exchange of potassium for sodium

One further possibility remains, however, concerning the localization of the secretory process This is the possibility that both reabsorption and secretion are functions of the proximal tubule, secretion representing a reversal of the reabsorptive process *In this case,* secretion and reabsorption could not occur simultaneously so that the increased chloride and bicarbonate excretion could be due only to decreased reabsorption It might throw into question the theory of direct cation exchange since it would leave room for reabsorption in the distal tubule of any secreted anion as the sodium salt However if we consider the effect of mercurial diuretics on the excretion of potassium it would seem very unlikely that secretion is simply *a reversal of the reabsorptive process I don't want to* go into detail now as to the nature of the effects of mercurials on potassium excretion Perhaps Dr Mudge would like to comment on this later I would like to refer to one type of experiment which is pertinent to this question If a dog is given an infusion of some potassium salt, so that a large excretion of potassium is obtained with an appreciable excess of excreted over filtered, and a dose of some mercurial diuretic is then administered, there invariably follows a marked drop in potassium excretion which usually reduces the ratio of excreted to filtered to well below unity Such a drop might be due to either decreased secretion or increased reabsorption If the effect of mercurial were on a single *alternatively* reabsorptive and secretory mechanism, a change in the ratio to one but not past it might be anticipated, since reduction to one would represent inhibition of the active process and past one an enhancement *of the active* process On this basis, it seems reasonable to conclude that the

reabsorptive and secretory mechanisms are functionally and presumably anatomically separate

To summarize briefly this rather involved argument there is reason to believe that two separate tubular mechanisms are concerned with the excretion of potassium With the assumption that the reabsorptive mechanism is located in the proximal tubule the secretory process is in all probability a distally located mechanism for exchanging potassium ions The role played by secretion in the excretion of low and moderate amounts of potassium is not clear but high rates of excretion are probably achieved by a combination of diminished reabsorption and increased secretion The factors controlling the rate of excretion are likewise not clearly understood but it is striking that the amount filtered is a relatively unimportant factor

I have rather neglected the subject of the excretion of acid and ꞏould like to return to it briefly before concluding There is some ꞏagmentary information concerning the intermediary processes ꞏvolved which should be brought up for consideration In their ꞏrk with the mechanism of urine acidification Dr Pitts and his ꞏociates (10) showed that the acidification could be at least par ꞏly inhibited *in vivo* by the administration of sulfanilamide They ꞏsented this as evidence that carbonic anhydrase played some central role in this process suggesting that the hydrogen ions were made available from carbonic acid formed by the hydration of CO_2 Davies and others (3) have suggested that the process of acid secretion in the stomach is analogous to that in the kidney and proposed a slightly different role for carbonic anhydrase They suggested that the hydrogen ions are derived from the reduction of iron porphyrins and that the carbonic anhydrase catalyzes the combination of CO with hydroxyl ions formed on the re-oxidation of the iron porphyrin One further observation may be of interest although its significance is not entirely certain We have found that acidification of the urine can be completely abolished in acidotic dogs with CO s as low as 10 mM per liter and blood pH below 7 3 by a dose of malleic acid of about 0 4 mM per kilogram Malleic acid you will recall is the cis isomer of fumaric acid. The system affected is not certain since a number of different effects have been ascribed to malleic acid A point of interest however is that malleate at a concentration of 100 mM per liter applied to the outer surface did not inhibit the secretion of acid by the isolated mucosa of the frogs stomach Be this as it may the most reasonable explanation so far offered would seem to be that secretion of

attributable to a fixed spatial arrangement of some enzyme or series of enzymes yielding hydrogen ions on reduction and hydroxyl ions on oxidation that the hydroxyl ions are combined with CO_2 to form bicarbonate which is returned to the interstitial fluid with the cation for which the acid has been exchanged

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DISCUSSION

Bradley To what extent do mercurial diuretics affect potassium excretion in the presence of low serum potassium concentrations?

Berliner I would say — and this is only a very rough approxima- [illegible]

what higher than that, say 50 to 75 µEq [illegible]

be much change If it is much higher than that initially, potassium excretion always goes down after the mercurial

Bradley How do you interpret that?

Berliner The changes after mercurial diuretics have been interpreted in two ways The obvious explanation would be a decrease in potassium transport in either direction (Mudge G H et al (8) (9)), reduced reabsorption and reduced secretion We have some data (the results have not been sufficiently reproducible to satisfy us entirely) which suggest that mercurials might *increase* reabsorption These findings originally gave us the idea that potassium might be secreted we found in several experiments (we were not giving potassium at the time) that following a dose of mercurial the excretion of potassium might go up or down but then reached some value at which it remained constant despite considerable changes in the amount filtered Now that suggested to us that there was some secretory mechanism working at a fairly constant rate and not dependent on the amount filtered This could produce the observed potassium excretion if all of the filtered potassium were reabsorbed say in place of non reabsorbed sodium so that the potassium coming out in the urine was only that secreted When that hypothesis paid off in our being able to demonstrate that there *was* secretion of potassium we thought it must have been a good idea (Berliner R W and Kennedy T J Jr (1)) I think now that there may be good reasons for questioning it It seems to be a rather extraordinary effect for an agent which usually depresses active processes to increase the reabsorption of potassium In any case there is the problem of why potassium excretion sometimes goes up and sometimes down If potassium secretion is a distal exchange for sodium it would not be unlikely that the rate of secretion might be related to the amount of sodium delivered to the distal tubules An effect of mercurials on sodium reabsorption in the proximal tubule might increase the sodium delivered to the distal tubules and consequently increase secretion of potassium

Schroeder Does not a mercurial sometimes increase the excretion of magnesium and calcium as well as potassium and sodium? That has been reported

Berliner Dr Farnsworth reported such changes We have not measured the excretion of calcium or magnesium

Schroeder I think she found chloride always came out but that the cations might be any one of those four

Berliner There is one experiment which might give conclusive evidence as to whether the effect of mercurial diuretics is diminished

Are we truly depressing reabsorption of potassium or are we stimu lating secretion?

Berliner Until you have ratios of excreted, filtered over 1, one cannot say We don't know any way of telling whether the change is due to changes in reabsorption or changes in secretion I would be inclined to say both, but that is purely personal prejudice and not based on any logical thesis Is that what you would say, Dr Mudge?

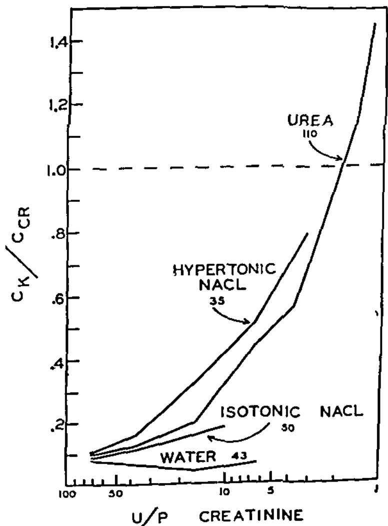


FIGURE 30

Mudge We have studied this problem with Dr Gilman With the possibility that two processes are occurring simultaneously, as has been indicated one cannot make a conclusive statement about what is happening in the tubules However, by studying a number of variables we have obtained data which seem best interpreted as showing that the increase in potassium excretion with hypertonic infusions is due to secretion In Figure 30 we have plotted the Ck/CF ratio against the U/P ratio of creatinine obtained during different types of diuresis The experiments with urea show that ratios, significantly over unity can be obtained without the administration of potassium These animals were hypertonic, we have obtained similar evidence of secretion with hypertonic sodium bicarbonate infusions, but our highest ratio with sodium chloride is about 0 8

Berliner Do you consider that an elevated urea makes the animal hypertonic?

Mudge Not directly, but in our experiments the serum sodium was always elevated We have not re studied the effects of urea with normal or low blood sodium levels As the figure shows, the results with urea and hypertonic saline are so similar that we have considered them both as representing the effects of hypertonicity It should be noted that, at any U/P creatinine ratio (10 for instance), more than twice as much potassium was excreted with hypertonic infusions than during isotonic diuresis Isotonic saline increased potassium excretion to about 20 percent of the filtration

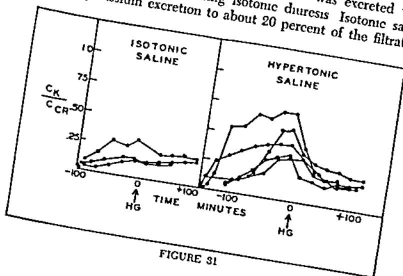


FIGURE 31

rate water diuresis on the other hand was associated with a slight fall in potassium output

In Figure 31 some experiments on the effects of mercurials given during saline diuresis are shown With isotonic infusions mercury produced little change in the potassium clearance However when the animals were made hypertonic the potassium clearance rose in every case and this was immediately depressed by mercury The experiments are comparable as far as the values for filtration rate serum potassium and total electrolyte excretion are concerned The concentration of serum sodium and the rate of potassium excretion were the only differences between the two sets of experiments

Thus there are two points which these experiments establish First hypertonicity increases the potassium clearance which under extreme conditions can exceed the filtration rate and secondly the increased excretion is readily reversed by mercurials We interpret these as indicating that the potassium excretion of hypertonic dehydration is due to secretion

Pitts Would you feel that the secretion of potassium is going on at all times and that the infusion of hypertonic saline depresses absorption perhaps proximal absorption if Dr Oliver will excuse the physiologist from talking about sites without looking Perhaps this reduction of proximal absorption could be an osmotic action uncovering what is going on all the time Would that at all be your conception?

Mudge If we were to say that hypertonic infusions depressed the proximal reabsorption of potassium because of the resulting increase in the rate of urine flow we would have great difficulty in explaining two related observations Why isn t there a similar effect from isotonic saline diuresis when urine flow and total electrolyte excretion are the same? And secondly if the increased potassium excretion were considered as depressed reabsorption then mercury would have to increase the rate of reabsorption For us at least it is difficult to conceive of a transport mechanism whose activity would be enhanced by an agent such as mercury

Selkurt What happens to the urine flow when you give mercury?

Mudge In four of five experiments it increased In one it did not

Shannon What is the total cation excretion that is sodium and potassium before and after mercury

Mudge In terms of molecular equivalents the changes in sodium are far greater than those in potassium For example in one of the

experiments with hypertonic saline sodium excretion was increased from 550 to 1400 μEq per minute while potassium fell from about 120 to 60 μEq per minute

Berliner Is that associated with a marked increase in chloride excretion?

Mudge Yes chloride excretion increased

Berliner We have wondered whether the effects of mercurials are exerted primarily on anion of cation reabsorption — whether it is chloride reabsorption that is primarily depressed and the increased sodium excretion brought about just because some cation has to go along or whether the situation is the reverse

Our original hypothesis is a very useful one but I'm now not so sure of its validity I would not be prepared to defend either position

There are some observations on man for which we don't have letailed studies which may be contributory Sometimes when a nercurial diuretic is given to a cardiac or cirrhotic there may be n increase in potassium and chloride excretion with practically o change in sodium excretion The sodium may go from 1 μEq per ay to 2 or 3 while the excretion of potassium may go up an extra 0 μEq per 24 hours

Schroeder Is that dependent upon the plasma level of sodium?

Berliner Practically all our cardiacs and cirrhotics who are edematous and retaining fluid have low plasma sodiums so it is difficult to say

Luetscher Have you observed tubular secretion of potassium in patients with chronic glomerulonephritis? Could not this mechanism become a very important factor in the elimination of potassium when glomerular filtration is much reduced?

Berliner We have studied potassium excretion in a number of patients (I might say that all the work I've been talking about has been done in dogs) You can demonstrate potassium secretion in normal man but it is hard to do because the injection of large amounts of potassium intravenously is so painful However if a patient with impaired renal function whether a cardiac hypertensive cirrhotic or nephritic has even moderate impairment of renal function it is easy to demonstrate potassium secretion Many patients with marked depression of renal function have what appears to be potassium secretion all the time with the qualification that the inulin clearance is equal to the filtration rate under these circumstances

Wesson Dr Anslow and I have recently done some experiments which confirm the findings of Drs Berliner and Mudge Like Dr Mudge we employed urea diuresis Our experiments bear on his data because where he was working at elevated plasma sodium levels we made considerable effort to keep the plasma sodium at normal or subnormal levels Consequently the occurrence of the excretion of potassium at a greater rate than it was being filtered cannot in these experiments be attributed to a high plasma sodium We felt that since the urea penetrates the entire body fluid space cellular dehydration does not exist The administration of urea does not necessarily interfere with a water diuresis While under a urea load you can have a clearance ratio of potassium to creatinine well in excess of 1 0 at the same time that the animal is water diuresing

Another thing which has been mentioned today is the change in urine pH when the rate of potassium excretion is rising We have performed the experiment in reverse When we administered a mercurial diuretic to an animal which had a previous high level of potassium excretion we got the same thing that Dr Mudge did a profound drop of the potassium excretion to very low levels but there was no change in the pH of the urine

From an entirely different line some data obtained by Dr M F Levitt in Dr Smith s laboratory bears out the concept of potassium excretion by the use of radioactive isotopes This is a field in which I don t feel qualified to offer criticism so I will just repeat the data Dr Levitt found the same effect which Dr W O Fenn has previously described without particular reference to renal function The procedure was this a single large dose of radioactive potassium was administered the dose was large in terms of activity but small in terms of total potassium in the injection Following the injection the plasma level of radioactivity rose abruptly then declined steadily During the hours following the injection Dr Levitt found that the ratio of activity to total potassium coming out in the urine was considerably in excess of the ratio of activity to total potassium present in the circulating plasma The only explanation thus far advanced to explain this finding is this the administered radioactive potassium will be picked up by highly vascular and metabolically active liver and kidney much faster than by other parts of the body Then while the activity of the liver and kidney are in excess of the rest of the body they will tend to lose their activity to muscle and other tissues If the activity of the potassium in the renal parenchyma is greater than that in the circulating plasma then the only way you

can get the more active potassium into the urine would be for it to come through the cells This we took as substantiating the concept of potassium secretion

Leiter Were any similar experiments carried out after increasing the tolerance of the dog to ordinary potassium?

Wesson The doses were well below those customarily assumed to have no biological effect

Pitts Under those circumstances the actual increase in the rate of excretion of potassium was very small?

Wesson It was not detectable

Berliner Was a large amount of potassium coming out in the urine?

Wesson No The experiments under which the animals might have been potassium loaded with large amounts excreted in the urine, were not done They might be very interesting

Berliner Have you any data on specific activity of any tissues in relation to specific activity of the plasma?

Wesson Dr Fenn has published a great deal of data on that subject I do not recall his exact figures Tissue analyses were not made in Dr Levitt's experiments

Mudge I believe that a similar finding has been reported with phosphate (Friedlander, H D and Wilde, W S Can transfer rate be calculated from urine isotope ratio? *Federation Proc*, 8, 51 *(1949)*)

Wesson I could not understand the data The same experiment which I have described for potassium was done by Dr Levitt with sodium, but no significant differences between plasma and urine were noted

Berliner The sodium in extracellular fluid does not differ very much from that of plasma so that there is no store of high activity

Wesson Since there is no place to store the sodium it is not real evidence against secretion of sodium

Darrow Dr Berliner, you might be creating an abnormal animal with high extracellular potassium You might load the animal and get some evidence However, the amount of filtrate is not so large as to give an answer Ordinarily potassium is not stored We tried to see if we could raise the muscle potassium by giving large amounts of potassium I think we gave water having a concentration of around 400 μEq of potassium per liter and the rats must have been excreting a urine having about 700 μM of potassium per liter The muscle composition changed but curiously potassium went down instead of up It went down about 10 percent in the

muscle and there was a slight increase in muscle sodium That would be in line with what you spoke of as the intracellular composition altering this mechanism This fits in with the increase in potassium excretion associated with alkalosis or after the animal has been loaded with potassium for several days

Mudge You gave potassium chloride and the muscle potassium fell?

Darrow Yes

Berliner Those calculations are based on the chloride space?

Darrow The tissue analyses are interpreted in terms of chloride being largely extracellular You can check the calculation in terms of sodium plus potassium in water but the evidence is that potassium left the cells

Mudge About 10 years ago didn t you report that muscle potassium rose when potassium was given?

Darrow We get muscle potassium to rise if serum potassium rises If the conditions are such that serum potassium does not rise in other words if the animals reach a steady state under a load of potassium muscle potassium will go down to normal or below normal Do you see what I mean? I don t believe we have gotten high muscle potassium except in the presence of high potassium in the serum (*Am J Physiol* 130 747 (1940) — See page 757)

Leiter Are these animals being dehydrated by the procedure?

Darrow [illegible] more potassium chloride [illegible] Then they refu[illegible] drated. We were trying to see if we could raise intracellular potassium while leaving the animals in a more or less tolerable state If we got potassium too high they decided they would rather live on body fluids than take the stuff we were giving them

Pitts [illegible] the means by whic[illegible] tissues?

Darrow I don t think we know anything about it as far as intracellular composition is concerned I analyzed some kidneys years ago but they gave me such a headache that I put the results in the wastebasket We have some recent preliminary analyses of kidneys of rats subjected to alkalosis or potassium deficiency There seems to be a considerable change in the ratio of chloride to sodium and potassium in these rats as compared to normals The changes suggest that there is more sodium in the cells I have a

whole row of bottles awaiting analysis when I can get a flame photometer working I believe we can get some methods permit accurate an[illegible]

[illegible]

enough to permit them to be interpreted to indicate that larger [illegible] look as though the [illegible] large changes The amounts of sodium and small amounts of potassium are staying in tubular cells I don t know how you would interpret such a finding in terms of absorption. It might mean greater reabsorption of bicarbonate or potassium I don t know what effect the change in cell composition has on enzymes

Leiter I don t see why renal reabsorption and secretion of potassium cannot be parts of the same mechanism dependent on the same transfer system but in dynamic equilibrium as to direction of transport If you depress one process the other might be stimulated the reabsorptive if you depress the secretory and vice versa

Berliner From what you say it seems to me that you are referring to two mechanisms this is in agreement with our hypothesis

Leiter I am suggesting one mechanism working in both ways at either end

Binkley It comes down to a matter of definition

Shannon Dr Binkley s system works two ways

Binkley There should be no difficulty in explaining such observation on the basis of such a system

Oliver May I ask why do you think the separation between the site of the mechanism is so great as from proximal to distal? Why could it not be upper proximal and lower medullary proximal? We know definitely that those two parts of the proximal have some metabolic differences Is there any reason to jump from proximal to distal?

Berliner No

Selkurt I have some data on the effect of renal venous obstruction in which you might be interested It appears to indicate the operation of a dual mechanism for reabsorption and secretion of potassium The procedure used is probably familiar to all since it is similar to that used by Blake and his associates (Blake W D Wegria R Keating R P and Ward H P Effect of increased renal venous pressure on renal function *Am J Physiol* 157,1 (1949))

The clearances of electrolyte by the right and left kidneys were studied separately The left renal vein was occluded in varying

degree so as to elevate the venous pressure unilaterally Under these circumstances we may use the clearance of the right kidney as control, thus eliminating the effect of systemic factors which presumably operate equally on both kidneys In Figure 32 the ratio between creatinine clearances of the left to the right kidney remains close to unity throughout an experiment in which the left renal venous pressure was raised stepwise Two clearance values were obtained prior to imposition of pressure in each stage of pressurization, and finally, on release of pressure The pressure levels are given at the bottom of the figure in centimeters of water Except in the third period of pressure elevation where the creatinine clearance dropped on an average of 10 percent below the average control figure, the filtration rate remained fairly constant

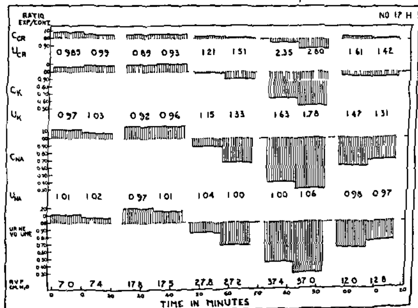


FIGURE 32

The sodium clearance ratio was well maintained until the second period of elevation where the clearance of sodium dropped to about 80 percent of control Finally during the period of highest pressure elevation, the sodium clearance dropped to about 30 or 40 percent of the control On release of pressure the sodium clearance came back to about 80 percent of control almost immediately The urinary sodium concentration ratio (U_{Na}) remained constant near

unity throughout this experiment Note also the very close correla tion between sodium clearance and urine volume the lowest figure It appears that reabsorption of sodium under these con ditions is perfectly isosmotic We have studied this effect in animals receiving isotonic as well as slightly hypertonic saline priming In the study shown here hypertonic saline solution was used Nearly all of Blake's studies were made on animals receiving slightly hyper tonic saline solution though he does mention a couple of experi ments in which he used isotonic solutions He does not give the relative data and one does not know what happened

Returning to potassium we find it does not behave like sodium During the first period of pressure elevation the potassium clear ance remained fairly high but began to fall in the second period was lowest in the third period of elevation and returned almost immediately to control upon release of the venous obstruction In most of our experiments the potassium clearance never drops as much as sodium or putting it another way the potassium urinary concentration ratio rises during this procedure from about 1 0 to as much as 1 7 We may assume that any potassium reabsorptive mechanism should resemble and behave like that for sodium Since potassium does not follow sodium and since it is somewhat concentrated in the urine it is possible that we are unmasking here a secretory mechanism which maintains a high urinary concentra tion and excretion of potassium despite increased reabsorption like that of sodium The other possibility of course is that po tassium is not as well reabsorbed as sodium under these circum stances I would welcome comment on these points Blake does not present data in his paper on the behavior of potassium

Bradley A similar disparity in the renal excretion of sodium and potassium ions appears in man when renal venous pressure is elevated by application of a tight abdominal binder under a pres sure of 80 mm Hg Dr Blake Dr Mudge and I have studied this response in five normal subjects and in seven patients with uncon trolled diabetes insipidus In nearly every subject elevation of renal venous pressure by abdominal compression resulted in a signifi cant and equal decrement in renal plasma flow and glomerular filtration rate The excretion of water sodium and potassium also decreased Water and sodium output usually fell markedly as in the patient with diabetes insipidus presented in Figure 33 As a rule urine flow decreased less than the sodium excretion so that the sodium concentration of the urine diminished both fell more than filtration and the ratio between output and filtration de-

time relationships Rats can be trained as it were to excrete water faster than normal Obviously that is in some measure a renal phenomenon but we thought it was in part a manifestation of extrarenal changes — although we didn't know what they were We did not think it had to do with increased adrenal cortical activity If I remember correctly the cytological evidence of Greep and Deane indicated that the administration of potassium will cause detectable adrenal changes interpreted as stimulation of the glomerulosa over a long period of time Even if that be true I think it would not necessarily follow that an adaptation of the renal tubule to handling potassium is due simply and solely to increased adrenal cortical activity Perhaps we can draw an analogy to what happens in a muscular system If an animal is subjected to severe exercise without any cortical hormone it will go into shock If it is subjected to exercise in the presence of cortical hormone it will adapt to the exercise and become capable of increased amounts of muscular activity This may be associated with an increased adrenal cortical activity but the adaptation occurs in the muscle and the cortical hormones while necessary to support the altered cellular activity, are probably not its direct cause Another example is that seen in *lactation Cortical hormones are necessary* for the synthetic processes of milk production and the amount of milk produced is to some degree dependent upon the amount of cortical hormone present Yet the hormones themselves have no lactogenic activity, i e they do not themselves stimulate milk synthesis They only *permit* other factors to do so I would guess that this same sort of thing might happen as far as the kidney is concerned as it adapts itself to handling specific substances That doesn t mean that the adrenal hormones do not effect specific kidney functions directly I am talking about adaptational phenomena

Luetscher I would like to back up Dr Gaunt s viewpoint Dr Deming and I have been using adrenalectomized rats in assaying desoxycorticosterone We thought at first that potassium excretion might be a useful end point but the results were so variable that we returned to the time honored effect on sodium excretion Even at doses fifty or a hundred times those necessary to produce detectable retention of sodium the increase in potassium excretion was small and irregularly related to dosage Extracts of urinary corticoids from a variety of patients have produced little or no increase in potassium excretion in a five hour period I wonder if the effect of DOCA is not primarily a sodium retention with a later displacement of potassium Perhaps our experiments are too

short to see the full effect Patients who receive an overdosage of DOCA for long periods lose potassium but there is usually an increase in serum sodium concentration or some edema at the same time

In studying patients with renal dysfunction due to disease the problems become even more complex In the nephrotic syndrome the retention of an excess of sodium in the body seems to be related both to a reduction in glomerular filtration and to an overactivity of the tubules in reabsorbing sodium even when the glomerular filtration rate is increased Assay of urinary corticoids in the severe forms of nephrosis shows an increased excretion of sodium retaining steroids At the same time the retention of sodium may produce a change in muscle sodium and potassium content similar to that observed in animals after prolonged overdosage of DOCA This leads to a situation in which one would have to balance the observations very carefully in order to distinguish what is primarily a renal action and what is a metabolic or displacement effect in the body cells

Heller Speaking about the effect of corticoids on potassium and sodium excretion I should like to mention a series of experiments which have just been completed (Heller H and Stephenson R P Effect of posterior pituitary extract and its fractions on renal electrolyte excretion *Nature* 165 *189 (1950)*) When small doses of pitocin or pitressin were injected into rats it was found that both posterior pituitary fractions increased the excretion of potassium after pitressin sodium excretion was depressed and after pitocin not significantly altered These results may be of interest as suggesting that renal potassium and sodium excretion are not invariably interdependent

Shannon What dose?

Heller Three milliunits per 100 gm rat injected subcutaneously Similar results in rats were obtained by O Schaumann (*Naturf u Med in Deutschland* 61 *192 (1939/46)*) who however used much larger doses (40 milliunits per 100 gms) His findings agree with ours insofar as increases of potassium excretion were noted after both posterior pituitary fractions and less sodium was eliminated when the pressor fraction had been injected On the other hand he found that the injection of his dose of orasthin (= oxytocic fraction) resulted in a markedly increased excretion of sodium in the urine

Gaunt We used only pitressin and the antidiuretic substance from serum We will have to try pitocin

cortical hormones in the control of water and sodium excretion is well worth while if for no other reason than that it may give a key to experimental preparations which in turn may permit the more critical studies of the kidney at normal water and electrolyte loads It is frequently essential in the initial phases of any investigation to produce wholly abnormal circumstances in order to clearly define the absence or presence of a certain mechanism However, one can take quite another view in terms of normal economy of the body at a later date For example we have a clear demonstration that the renal tubules both absorb and secrete potassium, still our data do not preclude the possibility that all filtered potassium is reabsorbed in the proximal segment, and potassium that appears in the urine under conditions of normal filtration rate, and normal electrolyte composition appears there as the result of secretory processes of the distal segment In other words control under normal potassium loads is a function of the distal process

Gaunt I can only say, both from our own experience and from that of helping write a recent review on the subject that it is almost impossible to make any definite statement about the effects of cortical hormones on salt and water excretion except in terms of a precisely defined experimental situation It is certainly easy in sorting out experimental literature to see that what happens in the animal is dependent upon various conditions of salt load, water load and — as Dr Shannon suggested — with the balance of steroid hormones present

We are accustomed to thinking of DOCA as a sodium retainer There are several reports showing that DOCA can increase sodium excretion if the conditions are right It is well known that cortisone may cause either sodium retention or sodium excretion depending on the conditions Dr Pitts and his group have even recently shown us that the adrenalectomized animal can actually reabsorb more sodium than normal in certain situations Generalizations are obviously difficult

Fremont Smith Are we not clinging to an old philosophical concept, now outworn of single causality? It seems to me that we will have to accept as basic to our thinking that the effect of any given stimulus on an organism is not a uniform response, but that the nature of the response is determined by the state of the organism I was so glad that Dr Gaunt and Dr Pitts, as well as Dr Wesson brought out the relativity of the effect of pitressin i e, that the antidiuretic hormone may be either diuretic or antidiuretic, the response depends upon the state of the animal when the hor-

of circumstances Instead of being bothered by the relativity of response we should try to specify the situation in terms of pertinently related sets of circumstances which influence or determine the response to the particular stimulus under study I believe it is both impractical and philosophically unsound to seek a single characteristic response to a stimulus

Shannon I

I think that ı

one knows al

system will always give the same result

Oliver But one never knows all that.

Shannon The point is when one gets variable results then one has to re-examine the system in order to determine or discover the additional conditioning factors That is not the same thing as saying that the same stimulus may produce different results in the same system at different times

Fremont Smith There is no point where one can get down below a concatenation of events If you go into the cell or into the nucleus or into the atom you are involved in the same situation! When you say you have to know all the variables and must hold all the variables constant in order that a given stimulus should invariably produce the same effect you are saying exactly the same thing that I am The stimulus has effect A under conditions A but the stimulus would also have effects B C or D under different conditions The concept of single causality has outworn its usefulness I feel it is more in accordance with reality and with the scientific method and less likely to get us into trouble if we accept and make part of our thinking the concept of multi causality and concatenations of events Our emphasis then will be to specify as many of the variables as we can indicating those which are controlled and to what extent they are controlled leaving also plenty of room for unknown variables Nevertheless I would like to emphasize again that I believe Dr Shannon and I are saying the same thing from different angles

Shannon I am not sure

monkey wrench into the situation and made Newtonian concepts invalid with respect to certain other areas That is exactly the situation here I cannot for a minute believe that the concept of clearance will not be fundamental with respect to certain problems but I also believe that there will be situations in which the present concept of clearance will provide at best only a first or perhaps a second approximation to the facts

I want to ask you to expose yourselves, and this seems a good chance to expose myself, to an idea which is quite contrary to the basic concepts by which many of us are operating I think it is generally believed today that the capillary glomerular bed is being used at close to maximum capacity most of the time There is evidence, of course, that glomerular filtrate does increase under certain circumstances The vascular blood flow through the kidney does go up and certainly good evidence exists that it can be sharply reduced as in anxiety reactions or in traumatic shock.

We have been talking about tubular reabsorption today and my concept has to do with tubular reabsorption It is generally agreed that when concentrated urine is being formed or when antidiuresis takes place there is an increased amount of tubular reabsorption It is assumed that such an increased tubular reabsorption is due primarily to an increase in the activity of certain tubular cells in all the tubules I would like to suggest the possibility of a simple and more flexible mechanism which would allow for changes in the amount of reabsorption by the tubules by varying the surface of the tubular cells to which the filtrate is exposed Thus if a given amount of filtrate were exposed or allowed to flow over a large tubular surface much more reabsorption would take place than if the same amount of filtrate were in contact with a much smaller area of tubular cells

The basic assumption underlying this hypothesis is that the normal blood flow through the kidney occupies only a fraction of the glomerular capillary bed at any one time and the fraction of glomerular capillaries which are open can be varied by renal vasoconstrictor mechanisms which allow for a *redistribution* of the functioning glomerular capillaries It is further assumed that the total amount of glomerular filtrate is approximately unchanged when the kidney shifts from producing a concentrated urine to a maximum water diuresis This I believe is in accordance with observations of Rehberg on man (Rehberg P B Studies on Kidney Function I The rate of filtration and reabsorption in the human kidney II The excretion of urea and chlorine analysed according to a

modified filtration reabsorption theory *Biochem J* 20 447-482 (1926)) and was borne out by the observations of my colleagues and me on man in which glomerular filtrate as measured by endogenous creatinine clearance did not vary significantly from a concentrated urine to a water diuresis

If we consider the mammalian glomerulus as reconstructed by Vimtrup (Krogh A The Anatomy and Physiology of Capillaries Revised Edition New Haven Yale University Press 1929) as given in the frontispiece we see that each glomerulus is composed of approximately 50 non anastomosing capillary loops Thus on theo

loop open in each But note that in such redistribution of blood flow from one glomerulus with 50 capillary loops open to 50 glomeruli with only one capillary loop open in each there need be no change in total blood flow i e a redistribution of a constant volume of blood flow through the kidney could provide two contrasting situa tions One in which the blood was evenly distributed through all glomeruli but with approximately only one capillary loop open in each In this case the filtrate would be distributed to all the tubules but only a small fraction to each This would result in a maximum reabsorption of filtrate and a concentrated urine In the second situation the same blood flow would be distributed only to approxi mately one in every 50 glomeruli Each of these however would have its 50 capillary loops wide open There would be the same amount of total renal blood flow as in the first situation and the same amount of total filtrate would be formed but in this case the filtrate would be distributed to only one in every 50 tubules and these tubules would receive approximately fifty times as much filtrate per unit of time as they receive in the first situation The filtrate would be exposed to only 1/50 of the total surface of tubular reabsorbing cells and the contact with these cells for each unit of filtrate would be of very much shorter duration This would be the situation of minimal tubular reabsorption and maximum diuresis

I have two figures to illustrate this hypothesis In Figure 34 on the left is shown a diagram of a glomerulus with only one capillary loop open to illustrate the suggested condition of all the glomeruli when a concentrated urine is being formed on the right is shown a diagram of a glomerulus with all fifty capillary loops open to illustrate the condition of these glomeruli which are open (only about one in 50 would be open) during a water diuresis Figure 35

illustrates the same two conditions on the left a diagram showing a large number of glomeruli with only one capillary loop open in each and on the right a diagram showing a large number of glomeruli with only a few open but with these few wide open i e,

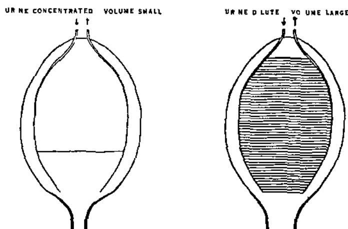


FIGURE 34

Diagrammatic picture of glomerulus on left with only one capillary open and on the right with 50 capillaries open It would require 50 glomeruli with only one capillary open to make as much filtrate as would be produced by one glomerulus with 50 capillaries open

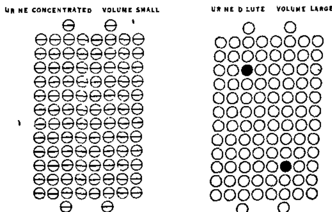


FIGURE 35

— f d tr b t on of blood flow in glomerular capillary bed
s
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50
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with all 50 capillary loops receiving the blood Thus by a redistribution of the same volume of renal blood flow in the one case a very limited flow through each glomerulus and in the other a maximum flow through only a few glomeruli, the same volume of glomerular filtrate would be delivered in the first instance to all the tubules and in the second instance to only about one in 50 tubules If such a mechanism does in fact exist one would anticipate a concentrated urine from the first situation because there would be maximum opportunity for tubular reabsorption while in the second situation diuresis would take place since all the filtrate would be pouring rapidly down only about one in every 50 tubules at any one moment

An hypothesis has virtue only if it will explain data more logically than any other hypothesis, if it provides for useful generalizations or if it acts as a challenge to new experimental work which otherwise might not be carried out I proposed this hypothesis before the American Society for Clinical Investigation in 1930 (Fremont Smith F, Fremont Smith M, Dailey M E et al Studies in Edema I The mechanism of water diuresis in man *J Clin Investigation* 9, 7 *(1930)*) without a real expectation that it would turn out to be correct but in the hope that it would stimulate new work Since that time I have discussed it informally whenever possible and have as yet to find what seems to me adequate evidence to dispose of this theory I would like to take a few minutes to show a number of physiological and clinical phenomena which perhaps can be correlated by this theory and which so far as I know are not yet correlated by any other theory

At present there is no theory to explain what happens in the kidney in the shift from a concentrated urine to a water diuresis other than the concept of increased reabsorptive activity of tubular cells nor is there any theory to explain the two contrasting clinical conditions i e edema without uremia and uremia without edema The proposed concept has the virtue of correlating the known renal pathology underlying these two contrasting conditions with their major differences in kidney function In other words one would predict that in a condition of edema without uremia the kidney is in a condition of constant maximum tubular reabsorption and yet has sufficient filtration surface to clear the non protein nitrogen from the blood and furthermore that the kidney is fixed in this condition at least temporarily From the viewpoint of the proposed theory one would expect the vast majority of the glomeruli to be functioning but almost none of them to be functioning fully i e, one would anticipate a diffuse injury involving all glomeruli but

allowing some blood flow through all or nearly all The actual pathology of glomerular nephritis, of nephrosis, and of amyloid disease of the kidney, in fact corresponds to just this condition All three show edema often without uremia In contrast is the pathology of the kidney in uremia without edema, as in the arteriosclerotic kidney Here the kidney is unable to concentrate the urine and a diuresis takes place continuously, in this situation the theory would require that there be a reduction of glomerular filtration surface to the point where the blood cannot be adequately cleared of non protein nitrogen but that those glomeruli which are functioning should be, in the main relatively normal, i e, be able to receive a maximum blood flow through all the capillaries Again this is in fact the case In the arteriosclerotic kidney there is a progressive loss of whole glomeruli – not a diffuse injury of all glomeruli As more and more glomeruli are destroyed, the blood flow through the kidney must be diverted to the remaining normal ones which will now have to open more and more capillaries in order to accommodate the renal blood flow and get rid of the non protein nitrogen This will mean that the filtrate is formed in progressively fewer glomeruli and is shunted down fewer and fewer tubules and one would expect and does in fact find a progressively increasing polyurea Eventually the filtration surface is insufficient to get rid of all the non-protein nitrogen, uremia progressively increases and the remaining glomeruli continue to function at maximum capacity Polyuria continues and edema does not take place The kidney at autopsy shows destruction of the majority of glomeruli but of the remainder many are not only normal in appearance but actually show dilation as if they were receiving constantly a maximum blood flow and making a maximum amount of filtrate It should be pointed out that a quite different clinical condition i e, congenital polycystic kidney shows the same clinical picture of progressive diuresis followed by uremia but never any edema, and again at autopsy shows massive destruction of the majority of glomeruli but those remaining appear quite normal and dilated

In animals it is well known that if the mass of the kidney is reduced by surgical excision, the urine flow does not decrease but increases! Thus if the kidney is reduced to one quarter its size the urine formed may be considerably greater than from the normal kidney This again could be explained on the assumption that the blood flow in the kidney of reduced size is now diverted through the relatively few remaining glomeruli and the filtrate formed pours into relatively fewer tubules with a resulting reduction of reabsorp-

tive surface and time for reabsorption with a resulting increased urine volume Diabetes insipidus according to this theory would be explained by the kidney functionally fixated in the condition of diuresis i e with blood flowing through a relatively few glomeruli which are wide open The antidiuretic action of pitressin would be explained not by an increased activity of all tubular reabsorbing cells but rather by redistribution of the blood flow in the kidney

only a minimum
glomerulus The
but in such small

volume that there is ample time for maximum reabsorption in each tubule

This concept therefore appears to offer a correlation of kidney function and pathology in the major clinical conditions of edema without uremia of uremia without edema of normal water diuresis of diabetes insipidus of the antidiuresis of pitressin and also of the increased volume of urine formed when the kidney is experimentally reduced in size

I would welcome the opinion of the group regarding this hypothesis Is there cleancut evidence at present to make the theory untenable? Can it be dismissed outright or on the other hand are there experiments that could be done which would either prove or disprove it? My own suggestion of an experiment would be in an unanesthetized dog with the kidney previously brought outside the abdominal wall to inject the renal artery with India ink This would be done in one group of animals during maximum water diuresis and in another group when a concentrated urine was being formed or after an injection of pitressin in each case during the first few seconds of India ink injection the pole of the kidney would be sliced off and immediately fixated Careful serial section recon

injected should show carbon in all capillaries of the glomerulus while in the animals producing concentrated urine all glomeruli should show India ink but only one or two capillaries in each glomerulus would be injected It would be necessary to slice off the kidney pole almost immediately after starting the India ink injection for there is nothing in the proposed theory which would eliminate "intermittance" i e during the formation of concentrated urine although only one capillary would be open in each glomerulus

at any one time the same capillary would not necessarily be open continuously Therefore after some minutes of injection all the capillary loops might show some carbon particles Similarly, in water diuresis with only one glomerulus in fifty open at any one time there would be the possibility — even the probability — that one glomerulus after *another would take its turn in becoming wide* open thus shifting the burden of tubular reabsorption from one tubule to another and avoiding fatigue or exhaustion of the cells which are actively reabsorbing water

This concept can be wrong but I have for nineteen or twenty years challenged everyone that I could from Rehberg on to bring forward any good evidence to show that this is impossible and I have not been able to have the pleasure of getting that evidence yet So I throw it back to you

Shannon I think Dr Forster has some data that might bear very directly on the problem

Forster I was originally interested in Richards techniques which *were developed of course for the direct examination of glomerular* and tubular activity Later when I turned to clearance studies I chose to work on the frog for the specific purpose of comparing *the results obtained by using clearance techniques on this animal* with those previously made by others using microdissection procedures One of the checks on the clearance technique was to see *whether such alterations in clearance would be obtained* as could be correlated with observations on glomerular intermittency observed directly in the frog The problems Dr Fremont Smith has *posed soon presented themselves Are variations* in urine flow accounted for by opening and closing of the glomeruli? Is glomerular activity an all or none phenomenon? Does a glomerulus *function all the time in its full capacity* or are there gradations of circulation through an individual glomerulus?

Dr Fremont Smith s Figure 34 a diagram of an individual glo *merulus presents the possibility* that blood in coming through the afferent arteriole can traverse all of these 50 channels one of the channels or any intermediate number of them Back in 1937 *or so I discussed* with Dr Shannon the possibility of using clearance techniques to examine the problem of glomerular intermittency and it was decided to measure maximal rates of glucose reabsorption (glucose Tm) by the tubules under conditions which radically altered glomerular filtration rates in frogs The concentration of glucose in the plasma was maintained two or three times as high as was necessary to insure saturation of the tubular reabsorp

tive mechanism In any single nephron unit then with the glucose plasma level several times higher than that needed to insure saturation it would be possible to reduce the filtration rate of its glomerulus by 50 percent or more without altering the rate of glucose reabsorption in the associated tubule We would expect then that if the situation illustrated in this slide prevails the filtration rate could fluctuate considerably without resulting in corresponding variations in the rate of glucose reabsorption As far as clearance determinations are concerned the glucose Tm would not vary directly with the glomerular filtration rate This was shown not to be the case That is the maximal rate of glucose reabsorption always exactly paralleled the glomerular filtration rate measured as the inulin clearance

Fremont Smith Did your experiment prove that you were using at any time all the capillaries in the kidney or that under a water diuresis which is quite different from a glucose diuresis you could not have a shift? My theory does not attempt to explain sodium chloride diuresis urea or caffeine diuresis but only water diuresis Different mechanisms are involved in these other forms of diuresis

Forster I just wanted to say that the urine flows in these frogs were varied at least tenfold even with the animals full of sugar by alternately taking them out of water and immersing them Filtration rates varied as well as urine flows Alterations in the filtration rates as great as tenfold in the frog were accompanied by exactly the same alterations in glucose Tm under these conditions

Fremont Smith If any glomerulus opened it did so completely?

Forster If it had been otherwise it would have been possible you see to close down 50 percent of the circulation reduce the filtration rate by 50 percent and have no change in the glucose Tm If this situation prevailed that is if instead of completely closing down glomeruli 50 percent of the channels through individual glomeruli were closed the filtration rate could have been reduced by 50 percent without necessarily altering the glucose Tm

Fremont Smith Then am I right that what your experiment proved was that under the conditions of the variations in glomerular filtrate due to this glucose situation glomeruli either opened or shut in an "all or none" way?

Forster That is right

Fremont Smith But it did not give any evidence as to the impossibility of their shutting and opening part way under other conditions

Forster That is entirely true

Shannon Dr Forster really should have emphasized that all these experiments were carried on pretty much with the same plasma glucose level He changed the glucose load by changing glomerular filtration rate not by changing plasma glucose

Fremont Smith Would it be assumed then from this that in the frog and in perhaps mammals if a glomerulus is open the blood flow per unit of time through that glomerulus is constant?

Shannon No sir

Fremont Smith It seems to me if you can have variations in the blood flow per unit of time through a glomerulus it gives opportunity for essentially the same variation in rate of filtrate flow down the tubules as suggested above

Shannon I think you have to draw on Homer Smith s observations with p aminohippurate for that if you accept the validity of his measurements I believe this can be done with safety since direct measurements by catheter techniques applied to the renal vein are in essential agreement Smith found in the human that one can change the blood flow up or down but there is no change in reabsorptive capacity This would imply that the reabsorptive surface has not changed

Grafflin Some years ago in a course in physiology for medical students many frogs were carefully (though still crudely) prepared for continuous observation of the living kidney in a selected microscopic field The phenomenon of intermittence in the glomerular circulation was regularly observed in the first 20 or 30 minutes after the preparation was completed If the frog s skin was then bathed with hypertonic salt solution all glomeruli became completely inactive within a very short time On the other hand if the skin was freely bathed with distilled water all glomeruli in the field soon attained what appeared to be maximal activity and retained it for prolonged periods

Fremont Smith Is that water diuresis associated with an enormous increase in glomerular filtrate?

Grafflin Measurements were never made

White Glomerular filtration comes up to normal when the frog is properly or normally hydrated If each loop were open there would be 50 times as much filtrate as on the left of your figure so that with all of them open there would be 6 000 cc a minute instead of 120

Shannon The frog does just that

White Dr Bott knows more about this than I do I don t know how Dr Richards feels about this since I have not talked with him

about it for a good many years but I believe he would say that when a frog is normally hydrated you don t see any intermittence You take the absence of intermittence as evidence of normal circulation the more normal he is the less likely is intermittence to occur

A number of years ago I did an experiment which in my naive way of looking at things had some bearing on this If you inject a little India ink into a dog s renal artery and clamp the pedicle four or five seconds later all the glomeruli will be injected Departures from that I am sure can be explained on chance distribution of the injected material You don t see intermittence You may see an uninjected wedge one whole interlobular artery or group of interlobular arteries may fail to receive it but where injected all of their glomeruli are injected. That is true whether the dog has been dehydrated or whether he had an abundance of water or was even given water by stomach tube before you do this I might say the same thing is also true in the rabbit He can be hydrated or dehydrated I believe recent work by Brod and someone else from Smith s laboratory accepts the view now that the glomerular filtration rate is not parallel with urine flow in the rabbit any more than it is in the dog or man (Brod J and Sirota J H Effects of emotional disturbance on water diuresis and renal blood flow in the rabbit *Am J Physiol* 157 31 *(1949)*)

Fremont Smith Would you give us the reference to your experiments on the dog?

White They were published (White H L Observations indicating absence of glomerular intermittence in normal dogs and rabbits *Am J Physiol* 128 *159 (1939)*)

Pitts Dr White I would like to ask if you think the frog abnormal every time it crawls out on a lily pad Does diuresis continue till the frog dries up?

White I don t believe he will stay on that pad long enough I believe he will see that he gets himself moistened The question of how dehydrated a frog can get and still be considered normal is of course open to debate I would like to turn the floor over to Dr Bott

Bott I don t pretend to be able to answer that question but I do know that when we were doing punctures on mammals there was the difficulty of finding the glomeruli on the surface Then there was another difficulty of collecting from some that were on the very surface It was easier to puncture those just slightly below and collect from them Dr Walker saw a number of them on the surface from which he could not collect and watched these for a

time never
however, t
and still be
This was n
it either, although saline infusions were being made

WATER REABSORPTION BY THE RENAL TUBULES

LAURENCE G WESSON Jr
Department of Physiology
New York University College of Medicine

Sodium falls into that group of substances whose rate of excretion by the kidney under normal or physiological conditions is subject to facultative control by the organism as a whole this control being exercised through functional variation in the level of activity of specific reabsorptive or excretory cells of the tubules or else through changes in the magnitude of the load delivered to the tubules Once a facultative control of electrolyte excretion is assumed the problem is to discover the sequence of events in the body whereby an increase in body sodium chloride is followed by excretion In the case of water excretion the primary event appears to be a change in the osmotic pressure of the extracellular fluid In the case of sodium the primary event appears to be a physical increase in the extracellular fluid volume or some fraction of it but the mechanism lying between fluid volume increase and increased electrolyte excretion is still unknown Whatever the nature of this mechanism its action remains almost unique among substances excreted by the kidneys since the full range of body salt control from complete retention to the excretion of the greatest endurable rate of salt ingestion is accomplished by an increase or decrease of only a few percent of the total amount of salt filtered at the glomerulus The ability to adjust body salt balance by fractional percentage changes in the total amount reabsorbed implies an extraordinarily sensitive mechanism and the immediate problem of salt reabsorption becomes that of determining how the percent tubular reabsorption can be varied within that small range Furthermore the importance of small percentage changes in total reabsorption greatly enhances the potential difficulty of studying sodium excretion for it is not surprising that a great variety of factors are known which will change at least temporarily by a few percent the percent of sodium reabsorbed by the tubules and therefore will produce as great a percent change as may be observed under so-called normal conditions by increased sodium chloride feeding A change in the diet innumerable

drugs renal oxygen supply renal venous pressure other excreted ions are some of these factors If we include with these endocrine and metabolic agents we have a group which might be called the minor variables to be distinguished from the major variable of filtered salt load with its two components concentration and volume The difficulty of controlling all of these variables is tremendous And any one or several of these minor variables although capable of affecting total salt reabsorption by only a few percent may be critical in determining salt excretion under physiological or pathological conditions

The mechanism by which sodium is reabsorbed by the renal tubules is almost certainly complex reflecting the sum of two or more separate reabsorptive mechanisms The evidence supporting the view that 3/4 to 9/10 or more of the filtered sodium chloride is reabsorbed in the proximal tubule of mammals is good In addition there appears to be good evidence for the existence of ion exchange mechanisms whereby hydrogen ammonium and potassium ions are secreted into the tubule in exchange for sodium ions reabsorbed such mechanisms together accounting for perhaps 1 to 2 percent of the total sodium filtered But there is also a philosophical reason for seeking the existence of still another reabsorptive mechanism for sodium and that is the belief that it is more probable to find the body controlling the excretion of sodium by large variations in a reabsorptive mechanism receiving small amounts of the filtered load of sodium than by small variations in a mechanism which reabsorbs the bulk of the filtered load We may then tentatively propose the following system The bulk of the filtered sodium is reabsorbed in the proximal tubule in large and variable amounts and without being under critical facultative control Distal to the proximal reabsorptive area but not necessarily located in the anatomical distal tubule is a distal reabsorptive area which has these properties a) its maximal reabsorptive capacity is equal to 10 to 20 percent of the normal filtered sodium load b) the magnitude of this distal process is subject to facultative variation by the body over a wide range c) the amount of sodium escaping from the proximal area is normally equal to or less than the maximal distal reabsorptive capacity Regions in which ion exchange takes place may be located either proximally or distally but it should be noted that if such exchange mechanisms exist in the distal tubule it is essential that an adequate supply of sodium remain to enter the distal tubule after proximal transfer is completed Variations in sodium excretion may then take place in either of 2 ways by

increasing the load of sodium escaping from the proximal process so that it exceeds the distal process or by decreasing the rate of distal reabsorption Either or both of these processes may be conceived to be under body control

It is by no means evident that it is possible to prove or disprove the existence of a third tubular reabsorptive mechanism It would seem theoretically possible to do so under direct examination by micropuncture but this may not be technically feasible so that we are at present limited to indirect methods Here the only technique which has offered any hope of success is a possible correlation between sodium and water reabsorption

According to all available evidence water is reabsorbed in large quantities in the proximal tubule The driving force for this reabsorption appears to be obligatory passive and due to osmotic pressure differences between intratubular and peritubular fluids which have been set up by the prior active transfer of solute If 50 percent of the filtered solute measured as osmols is transferred then 50 percent of the filtered water is capable of moving until isotonicity across the epithelium is restored Conversely if 50 percent of the water diffuses from the proximal tubule then it follows that at least 50 percent of the osmols has been transferred also Since sodium salts account for 90 to 95 percent of the total filtered osmols the amount of water transferred measures as a first approximation the amount of sodium transferred According to the micropuncture studies the proximal tubular fluid remains very nearly isosmotic with plasma as far down as the investigators have been able to go In this case the measure of solute transfer is a precise one It does not follow however that osmotic equilibrium across the proximal epithelium is so nearly complete under more physiological conditions The fluid reaching the end of the proximal tubule may be quite hypotonic to plasma due to the inability of water to keep pace with solute transfer

Accordingly these possibilities may exist at the end of the proximal tubule

1 A small volume of fluid representing virtually complete transfer of electrolyte and isosmotic transfer of water
2 A larger volume of strongly hypotonic fluid representing virtually complete transfer of electrolyte and incomplete transfer of water
3 A large volume of fluid nearly or completely isosmotic to plasma and therefore carrying considerable quantities of electrolyte

drugs renal oxygen supply renal venous pressure other excreted ions are some of these factors If we include with these endocrine and metabolic agents we have a group which might be called the minor variables *to be distinguished from the major variable of* filtered salt load with its two components concentration and volume The difficulty of controlling all of these variables is tremendous And any one or several of these minor variables although capable of affecting total salt reabsorption by only a few percent may be critical in determining salt excretion under physiological or pathological conditions

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increasing the load of sodium escaping from the proximal process so that it exceeds the distal process, or by decreasing the rate of distal reabsorption. Either or both of these processes may be conceived to be under body control.

It is by no means evident that it is possible to prove or disprove the existence of a third tubular reabsorptive mechanism. It would seem theoretically possible to do so under direct examination by micropuncture, but this may not be technically feasible, so that we are at present limited to indirect methods. Here, the only technique which has offered any hope of success is a possible correlation between sodium and water reabsorption.

According to all available evidence, water is reabsorbed in large quantities in the proximal tubule. The driving force for this reabsorption appears to be obligatory, passive, and due to osmotic pressure differences between intratubular and peritubular fluids which have been set up by the prior active transfer of solute. If 50 percent of the filtered solute, measured as osmols, is transferred, then 50 percent of the filtered water is capable of moving until isotonicity across the epithelium is restored. Conversely, if 50 percent of the water diffuses from the proximal tubule, then it follows that at least 50 percent of the osmols has been transferred also. Since sodium salts account for 90 to 95 percent of the total filtered osmols, the amount of water transferred measures, as a first approximation, the amount of sodium transferred. According to the micropuncture studies, the proximal tubular fluid remains very nearly isosmotic with plasma as far down as the investigators have been able to go. In this case, the measure of solute transfer is a precise one. It does not follow, however, that osmotic equilibrium across the proximal epithelium is so nearly complete under more physiological conditions. The fluid reaching the end of the proximal tubule may be quite hypotonic to plasma due to the inability of water to keep pace with solute transfer.

Accordingly, these possibilities may exist at the end of the proximal tubule:

1. A small volume of fluid representing virtually complete transfer of electrolyte and isosmotic transfer of water.
2. A larger volume of strongly hypotonic fluid representing virtually complete transfer of electrolyte and incomplete transfer of water.
3. A large volume of fluid, nearly or completely isosmotic to plasma and therefore carrying considerable quantities of electrolyte.

4 A varying combination of the other three

Whether or not water may be used as an index of sodium reabsorption depends upon which of these conditions is the true one

A *second fraction* of water reabsorbed is that *represented by the production of a hypertonic urine* This might represent in man 3 to 5 cc per minute and necessarily requires the direct expenditure of cellular energy as osmotic work The locus of this activity may be tentatively referred to the distal tubule A *third fraction* of water reabsorption is that which appears as an increase in urine flow during water diuresis or as a result of interruption of neurohypophyseal function and represents about 15 percent of the total filtered It is generally assumed that this water arises from the depression of a *specific reabsorptive mechanism brought about by a decrease in* the concentration of circulating antidiuretic hormone Two theories have been advanced to account for this *facultative water fraction*

According to one theory the facultative water arises from the thin limb It is postulated that an abundant hypotonic fluid enters the thin limb Under the stimulus of the antidiuretic hormone water passes through the epithelium of the thin limb until osmotic equilibrium is attained and a scanty fluid enters the distal tubule to be further concentrated by the active reabsorptive process In the absence of the antidiuretic hormone the thin limb remains impermeable to water so that an abundant hypotonic fluid enters *the distal tubule in excess of the distal reabsorptive capacity* and escapes from there into the bladder

According to the second theory the facultative water is reabsorbed actively by the same distal mechanism which produces a hypertonic urine In this regard it is a simplification over the foregoing In the absence of stimulation by the antidiuretic hormone active reabsorption is depressed so that an increased volume of fluid escapes into the collecting tubules It is not essential to this theory that the fluid entering the distal tubule and from which the facultative water is abstracted be isotonic although proposing that the tubular urine comes to osmotic equilibrium in the thin limb is an intriguing auxiliary hypothesis Nevertheless in order that water reabsorption may serve as a key to the existence of an additional sodium reabsorptive process it must have just these properties that osmotic equilibrium be attained in the proximal tubule or thin limb (and it makes no difference which) thus affording a measure of the amount of sodium reabsorbed in the proximal tubule And further that the facultative water be reabsorbed actively in the distal tubule thus affording a measure of the volume of electrolyte

entering the distal tubule Subtracting the amount excreted from the amount entering measures the magnitude of reabsorption by the distal tubule Measuring hydrogen, ammonium and perhaps some fraction of potassium excretion will determine the sodium reabsorbed by transfer If this or an analogous situation does not describe the behaviour of water, then water cannot be used as a measure of sodium movements in a complex reabsorptive system of the type described The mechanism of water reabsorption must be solved before much further progress can be made with sodium Until it can be determined whether there are one or two specific sodium reabsorptive processes, and if multiple whether they can be measured, investigations on such things as the relationship of filtration rate and plasma concentration of sodium to sodium reabsorption, the actions of mercury, of pituitary and adrenal hormones, of osmotic diuretics and other ions and the mechanism through which changes in body salt content are corrected by changes in the rate of excretion must rest upon a very tentative basis

There are so many problems associated with salt and water reabsorption and excretion that I would prefer to save them for general discussion I would like to conclude this introduction by listing some viewpoints which have been conditioning our approach to the problems associated with water and salt excretion

We feel that investigation of the mechanisms of sodium and water reabsorption should be initiated in the following order

1 Nature of the components of the water reabsorption system
2 Nature of the components of the sodium reabsorption system
3 The relationship of load, and its two components, concentration and volume to reabsorption by each component of the sodium reabsorption system Of particular interest is the relationship of filtration rate, the volume factor
4 Which of the variables relating to which component of the sodium reabsorption system is normally employed by the organism to adjust body sodium content to variable rates of sodium ingestion
5 The details of the mechanism by which the body controls this variable or variables
6 Elucidation of the effects of other factors, such as pH variable anions, hormones etc upon sodium and water excretion in terms of their relation to specific components of the sodium system

DISCUSSION

Pitts There is a question which has worried me all the way through this presentation I would like to ask all of you your opinions and Dr Bott especially We all have the tendency I think to accept the work of Walker Bott Oliver and MacDowell as the basis or starting point for the interpretation of all data on salt and water excretion Their experiments on the mammal were performed presumably under a given set of constant conditions which were neither those of marked osmotic diuresis nor those of water diuresis I wonder if Dr Bott would care to comment whether she feels that it is valid to assume that fluid is absorbed isosmotically in the proximal segment and that 66 percent or more of the filtrate is returned to the blood stream in that segment under conditions of water diuresis and osmotic diuresis as well as under the conditions of her experiments

Bott I must admit as we certainly had to from the beginning that these were not absolutely normal animals in that they had to be anesthetized Certain procedures had to be done on them in order to be able to take the samples and I think that we do have to keep that in mind constantly in interpreting the results I believe we have all admitted that However I feel that they are certainly a good basis from which to start I dont know what other one would be better As far as the osmotic pressure is concerned I think that surprised us as much as it did anyone to find these low osmotic pressures of tubular fluid all the way down and by the way I believe the three determinations on the distal tubule are perfectly real results They are very often not considered because we were hesitant to put too much stock in them having only three that Dr Walker felt were reliable collections Just how much the anesthetic might influence the results I cannot say for sure I have been asked something about the chloride determinations and whether they fit in with the low osmotic pressures I feel there is nothing in those chloride values that interferes with the acceptance of those osmotic pressures because the calculated osmotic pressure of the known constituents still leaves some room to spare

Pitts I am afraid I created the wrong impression by my comments I did not expect you to defend those specific experiments because we all accept them The fact that disturbs me is not that those experiments were performed necessarily under abnormal conditions I would consider they were a whole lot more normal than are those experiments performed under high osmotic loading But

can we assume that the same type of proximal osmotic relationships will obtain under osmotic loading? Also can we accept the premise that the proximal tubule cannot perform osmotic work on water necessarily? Is that absolute?

Bott I think the only way to be sure about the osmotic loading question is to try it in experiments of this kind I think that there is no reason why they cannot be done with time and trouble though Dr Walker is not working in the field just now

Selkurt Will you clarify what you mean by osmotic loading? Extraneous substances like mannitol?

Pitts Any type of osmotic loading whether by urea sodium or mannitol I am getting down to the point of asking Dr Bott whether she contemplates at all providing the data herself

Bott Some of our experiments were run during sucrose injections so there must have been quite a bit of sucrose present in the tubule fluid Unfortunately I don t remember which were which but in going back over my notes I may be able to find out where we did have a lot of sucrose present and if we still got the low osmotic pressure [1]

Shannon Could Dr Bott tell us the order of magnitude of the osmotic pressure difference?

Bott In many cases it was within experimental error of the osmotic pressure of the plasma through the first third or half I think of the proximal tubule There was one definitely lower one I think I would hesitate to say how much lower but it was definitely lower It would seem to be out of line with the others Those three on the distal tubule were still lower than most of those in the proximal tubule Then when the osmotic pressure of bladder urine was taken that was very high comparatively Of course there is a big gap in between The part that struck me in that too is that the chlorides were higher than the chloride of the plasma in the proximal tubule where the osmotic pressure was about equal to that of plasma The chloride in the urine was lower than that of plasma in spite of greatly increased osmotic pressure In other words the increase was not due to an increase in chloride

Wesson What was the osmotic pressure in the distal tubule in the three or four samples you obtained?

[1] (A search of my notes revealed that osmotic pressure of tubule fluid was not determined after sucrose injections but that tubule fluid osmotic pressure was low in the p of considerable glucose after phlorhizin)

Bott It was plotted as related to plasma I cannot remember exactly but I think it was 20 percent lower I would have to check that

Wesson Further on down the urine was found to be hypertonic?

Bott That is right

Wesson Suggesting that water reabsorption took place somewhere between the point of puncture and the bladder

Bott Early distal

Shannon We always talk about the mammalian kidney being characterized largely by its ability to form a hypertonic urine This potentiality is strikingly limited Under the most extreme conditions of dehydration without adding unusual constituents it is unusual for freezing point depressions to overrun about 1 3 1 4°C freezing point depression That occurs at low flows of urine in the dog in the order of 0 2 milliliters per minute Should one accept the data of Drs Bott and Walker as indicating isotonic tubule urine in the proximal segment then in the normal animal without any osmotic loading perhaps one might expect there to be osmotic pressures that are significantly lower than those obtained in the rat It is pure speculation

Pitts Getting back to your own experiments with dogs in diabetes insipidus when they were dehydrated they did form as I recall urine which was considerably hypertonic —

Shannon Not considerably The plasma reached a freezing point depression in the order of 0 7°C the urines were a maximum of about 1 0°C While all the dogs studied had diabetes insipidus as ordinarily described some had some residual posterior pituitary tissue The striking thing as far as we were able to determine was the lack of correlation between the amount of apparently normal tissue remaining and the animals ability to produce hypertonic urine

Pitts I would like to ask Dr Wesson and Dr Mudge if they have any indication of what the relative magnitudes of absorption of sodium or chloride might be in either the proximal or the distal segment under normal conditions

Wesson We have no direct experimental indication of these magnitudes Any estimate that we might make would be only a deduction from a current working hypothesis It is our feeling that if tubular water reabsorption has certain properties then we have a useful clue to the magnitudes of proximal and distal sodium reabsorptive processes If for instance the fluid reaching the end of the proximal tubule is measured by the urine volume of diabetes

insipidus and if further this fluid is isotonic with plasma then the amount of sodium reabsorbed in the proximal process is readily calculated

Shannon May I ask a question? Any assumption is reasonable if it leads to the design of a critical experiment However in retro spect you say the first assumption is that the tubule urine at the end of the proximal segment is isotonic or essentially so and the total argument really from there on depends upon that one point

Wesson The little evidence available seems to support that view Otherwise it is only a useful working hypothesis which we wish we could subject to experimental test

Pitts Basically it goes back to Dr Bott s demonstration that half way or more down the tubule it is isotonic By extrapolation we maintain that it is still isotonic at the end That is the reason for my initial question Is that reasonable?

Wesson Isotonicity throughout the proximal tubule seems reason able under the abnormal circumstances of heavy osmotic loading At least the amount of water which must be transferred to assure isotonicity of proximal urine with plasma is quite small and there is no reason to suspect that the osmotic agent opposes a barrier to equilibration However we do not feel justified in extending this reasoning to normal conditions where the osmotic load is very small

I would appreciate Dr Shannon s comments on this series of experiments Suppose it is observed that the total increment in urine flow when antidiuretic hormone is withdrawn from animals under a heavy water load is constant and independent of the magni tude of a simultaneous osmotic load Could that be interpreted as evidence that the volume of the increment measures a specific reabsorptive process?

Shannon I should think so However the setup is so complicated that it is difficult to make use of the information Let us start with *a simple system with a normal glomerular* filtration rate and remove the antidiuretic hormone either operatively or through the adminis tration of water One obtains a urine high in volume essentially low in electrolyte concentration I believe everybody has assumed until recently that this situation is due to the absence of the posterior pituitary hormone in which case the distal segment does not actively reabsorb water What appears as urine may be essentially the same type of fluid as leaves the proximal segment I am wonder ing if we can take the newer information that we have now and add onto such a rather simple working hypothesis If we accept Dr Wesson s view and accept as probable an additional series of

reabsorptive processes we may complicate the working hypothesis unnecessarily We have before us as of today undoubted evidence of secretion of potassium I wonder if it is not reasonable to suppose that this may not be unique for postassium It is conceivable that the nephron can also secrete sodium I find it not at all difficult to accept the possibility

With such a system it is possible to assign to the proximal the reabsorption of sodium potassium and water and assign to the distal segment the further reabsorption of water and the final definition of electrolyte composition to three processes that take place in the distal segment the secretion of potassium the secretion of sodium and the adjustment of the hydrogen ion concentration through a hydrogen ion mechanism If you accept the reasonableness of such a conception an experimental hypothesis emerges which is capable of being tested in a fairly direct way

Pitts It seems to me what we need basically is to be able to measure either the proximal or the distal reabsorption of either sodium or water I see no way under the sun to do it to do either one of those

Wesson An advantage to a four variable system is that you can explain almost anything with it

Shannon That is the difficulty

Bott I would like to correct an impression that I may have made a while ago when I was saying that I thought that these things can be done I was not thinking particularly that I would do them However I do have available now an ultramicromethod for inulin I felt the necessity for having a measurement of water reabsorption in all of these things In fact if we had had that inulin method at the time we certainly would have used it We also have now the sodium method and a very excellent chloride method and I am beginning on a potassium method which I have been thinking about for years and to which I am finally getting around It may be that with group work we can get along much faster than I could by myself and I don t pretend that I am promising to do all this myself but I think we have a number of methods available for doing work of this kind that we did not have a few years back With all the preliminary work that Dr Walker did on finding out what animal was best and what he had to do to get the best conditions — with all of that accomplished I think that it should not be too difficult to go on with some punctures on mammalian kidneys The thing I have been wondering is whether some preliminary information on the amphibian kidney would be good at all You

remember that some of these things show up in the amphibian kidney fairly well For instance that mounting up of chloride in the proximal tubule — we thought perhaps it was not even real in the amphibian because it was comparatively small Yet if you study the diagrams closely you will see that in many cases it went up almost 20 percent higher than the concentration of the plasma and in the mammal that was exaggerated and the tubule fluid at the same time was approximately isosmotic Of course I realize you don t get hypertonic urine but I wonder whether we could learn anything from the amphibia before going to mammals

Fishberg I wonder if one could not further the knowledge of tubule physiology by a study of chronic renal insufficiency in man There you have in man a urine isosmotic with plasma and the freezing point does not vary at all It is not glomerular filtrate because various constituents may be present in higher concentrations than in the plasma but it is isosmotic I have seen such patients in the last days of life with a urinary volume of 50 cc in 24 hours and urea in the blood ten times normal and the urine was still isotonic with the plasma This urine must be very similar to the fluid issuing from the proximal tubule I wonder if a study of such patients would not reveal a perpetuation of one stage of urin formation So far as I know it has never been studied in detail

Shannon There is another point from quite a different point of view that might be mentioned A fair amount of work has been done on the composition of fresh water and marine teleost fish urine By and large the freezing point depressions are low In such kidneys absorption of solute proceeds at a more rapid rate than absorption of water and the rate of flow down the nephrons is rather slow the urine flow being in the marine teleost about 4 cc per kg per day This is the result of activity of a single proximal segment in the healthy sculpin During diuresis as the result of skin injury the fish begin to excrete sodium chloride and urine is formed at a very high rate I don t recall the composition of such urines

Wesson Dr Anslow and I have some experimental material which might be of interest in this general problem We have attempted the experiments which I described earlier to Dr Shannon that is the superimposition of a water diuresis on top of an osmotic diuresis In several experiments the increase in urine flow due to the water diuresis is about the same size as the water diuresis in the same animal when not under an osmotic load

Fremont Smith Could you get any greater urine flow than that which occurs with a maximum water diuresis?

Wesson No, we could not get a larger increment than the control The relative constancy of the results thus far are inclining us to the view that the water of water diuresis measures some fairly specific reabsorptive process I am interested in criticism of this viewpoint since acceptance or rejection of this type of data could be critical to the interpretation of other experiments

Pitts Was there any change in osmotic pressure of the urine during the superimposed water diuresis?

Wesson The control urine, during osmotic diuresis alone, was essentially isotonic With the onset of water diuresis, it became strongly hypotonic

Pitts What is your concept then? Is it that the fluid delivered into the distal segment is hypotonic and water is absorbed to bring it to isotonicity?

Wesson We cannot see anything in these experiments which would answer that question For no other reason than simplicity, we have been inclined to the view that the water of water diuresis is reabsorbed during oliguric conditions in the same region where the urine is concentrated Until we have data to the contrary, it is simpler to think in terms of 2 fractions of water reabsorption rather than three

Gaunt How did you induce a water diuresis under these circumstances?

Wesson We put water into an osmotically loaded animal The water was kept in the body by the continuous administration of pitressin When urine flow and composition stabilized, the pitressin infusion was stopped

Shannon What was the urine flow before the water diuresis?

Wesson Eighteen cc per minute and it rose to about 23 cc per minute

Shannon Did the change of osmotic pressure reflect just that difference?

Wesson Yes

Mudge Did the sodium excretion change?

Wesson There was frequently a slight drop in sodium excretion when the pitressin infusion was stopped

Shannon I did not think that that experiment was not feasible because I assumed you did it when you described the result I believed it was difficult to devise an experiment to isolate a single segment for examination on the functional basis If one accepts this experiment as a valid prototype of a number of experiments which you probably are going to do, would not they argue for a hypo-

tonicity of tubular urine before it reaches the site of secondary water reabsorption?

Wesson It could argue for hypotonicity at that point but I don t think it is a necessary corollary

Shannon No

Wesson If we exclude from consideration the secretion of water it is evident that at some point the reabsorption of solute sodium chloride mostly from the tubular urine has outstripped water reab sorption in order to form hypotonic urine On the basis of the few experiments which we have done the hypotonicity seems to be measured better in absolute terms than in terms relative to some other variable such as filtration rate osmotic load or total sodium or water reabsorption Hence we would infer tentatively that the magnitude of water diuresis is more closely related to a certain small fraction of solute than to a hypotonicity developing from the beginning of proximal reabsorption of solute Whether this small fraction of solute and the water are normally reabsorbed in the same segment or whether on the contrary the solute is normally reabsorbed in one region leaving a hypotonic fluid to be facultatively reabsorbed in a lower region is something we have no idea of

I think that Dr Shannon s data on the effects of water and osmotic diuresis on urea clearance are still pertinent with their suggestion that the permeability to urea of that portion of the tubule normally reabsorbing facultative water is low It is tempting to infer that this may indicate a low permeability to water also whence it would follow that the normal reabsorption of facultative water is an active instead of a passive process

Berliner We were rather disappointed in the fish kidney for the

in the urine

White You spoke about the assumption of a more complete reabsorption of sodium and potassium both as I understood you in the proximal tubule assuming quite hypotonic urine and then the distal putting back whatever salts were needed I was not clear how you proposed to put this to experimental test

Shannon Dr Wesson gave one such experiment which would fit in with the concept that there are two general areas in the nephron one that is primarily concerned with reabsorption of sodium and potassium and one distal to that reabsorptive area

and influenced by the osmotic gradient where a sizable proportion of water is reabsorbed One may speculate that reabsorption of water is not complete to the point of osmotic equilibrium The more distal portion of the nephron may be assigned three specific functions the active secretion of potassium perhaps the active secretion of sodium and third the facultative reabsorption of water

White Even though the fluid issuing from the proximal tubule were quite hypotonic it still would not necessarily follow that all of the sodium is gone

Shannon Oh no!

White There could still be more than enough sodium to account for what appears in the urine I don t see why we have to have any secretion of sodium

Shannon One does not have to assume it However it is difficult to be certain it does not occur and equally difficult to design an experiment to demonstrate the presence of such secretion

White Until there is some reason for believing it does take place we can only speculate that it might Of course that is permissible

Pitts I think you have cleared up the question I directed at Dr Mudge Dr Wesson I was not questioning necessarily isosmosity of the proximal fluid but rather the argument that since the urine is isosmotic the distal tubule is doing nothing It may very well be absorbing water and whatever else it absorbs more or less isosmotically too As your own experiment would indicate it is absorbing some five to six cc of water What you get out in the urine is not what entered the distal tubule volume has been reduced by activity of the distal tubule and since osmotic pressure is unchanged solute must be removed. Both processes apparently occur in osmotic diuresis

Mudge I would like to ask Dr Wesson how he explains the fact that in acute experiments such as those with saline infusions the increased excretion of sodium is almost always associated with an increase in the amount reabsorbed The amounts filtered reabsorbed and excreted all increase I do not see how the increased excretion can be explained on the basis of saturation of a limited reabsorptive mechanism

Selkurt Perhaps our work may throw some light on this question We infused dogs with hypertonic sodium chloride and related the excretion to the load in the sense it can be done for glucose This was an attempt to see whether an active mechanism such as that postulated might be saturated

lines) and sodium excretion (lower lines) expressed in millimols per minute are plotted against the filtered loads of sodium expressed in millimols per minute The solid circles or triangles represent the ascending loads as we rapidly infused the sodium chloride The first two values on the left are the control values After stop-

animal No 26 where reabsorption is less during the phase of decreasing load

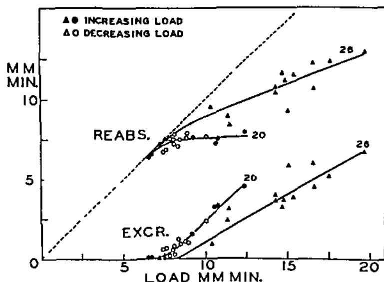


FIGURE 36

Figure 37
plotted as ır
lımıtıng fact
but in others it continues to rise continuously

Shannon Is there anything peculiar in the way the ex
on dog 26 was set up?

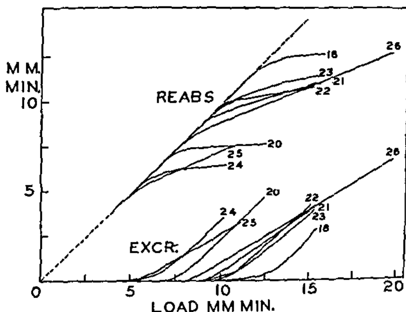


FIGURE 37

Selkurt No, dog 25 shows a similar response We are wondering if this does not indicate some limitation of the transfer mechanism If it does, of course all the explanations that might be given here would have to include the possibility of changes in hormonal balance resulting from the hypertonic infusion

Shannon What was the time relationship in the experiment?

Selkurt This has to be done rapidly As you know, when you load with sodium, sooner or later the plasma sodium level begins to stabilize It is very difficult to increase the sodium load sufficiently o produce elevated plasma sodium concentrations Each experi nent takes about two hours about an hour for each phase

We infuse five percent sodium chloride at a rate of about 10 cc per minute giving from 500 cc to 1 000 cc Then we half the infu ion and follow the descending relationship in the same manner

Mudge Were any dogs studied twice?

Selkurt No These dogs were anesthetized with pentobarbital and sacrificed at the end

Wesson Dr Selkurt and I have compared notes and we find that our data agree very well He has been studying the earlier phases of one of these infusions and we have been interested in some of he later phases These are curves drawn from pooled data on

several dogs with several experiments on each dog (Figure 38) All of the data has been put together in order to give some idea of the order of variation observed The figures do not represent the consistency with which data can be obtained in a single animal

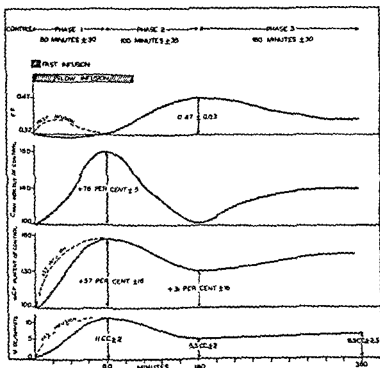


FIGURE 38

Average values for urine flow (V) filtration rate (uCF) renal plasma flow (CPAH) and filtration fraction (F F) following expansion of the extracellular fluid volume in 18 experiments on six normal dogs Numbers are means and mean deviations from the mean Before averaging the individual curves the time scales of the experiments on each animal were adjusted so that their inflections coincide with the average inflection times for all experiments

since repeated experiments on a single animal will show far less variation *Our procedure was to administer saline in the form of Locke's solution*, and as fast as the animal excreted water and electrolyte in the urine we put back into it more fluid of the same volume and composition as the urine so that plasma volume and composition remained essentially unchanged from the beginning to the end of the experime [illegible]

about sixty minutes after beginning the infusion we find that the increase in urine flow (V) is of the same order of magnitude, in general as the increase in creatinine clearance (wC_F), but averages somewhat less Since this urine is rich in sodium, *most of the* increased filtered sodium load has appeared in the urine Consequently, if we plot filtered load against reabsorption during this period, we find a flattening out, just as Dr Selkurt, suggesting a resistance to increased reabsorption If, however, one follows these dogs 6 or 8 hours after the infusion, then one finds a different picture The creatinine clearance goes through a dip and about 5 hours

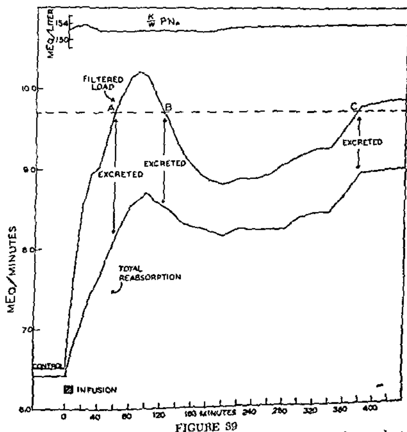


FIGURE 39

The filtered load of sodium ($k/w\ P_{Na} \times wC_F$) and rate of total sodium reabsorption in two dogs (two experiments per dog) following fast infusion of Locke's ...tion in two dogs (two experiments per dog) following fast infusion of Locke's ... The interval between load and reabsorption measures the magnitude of ... increasing tubular reabsorption ... B and C Glomerular ... than 2 mEq/L The ... end to Figure 38

after the beginning of the experiment has risen again very nearly to the maximum (Figure 38) The late urine flow, however is quite different from what it is at the peak of creatinine clearance early in the experiment

Figure 39 shows that sodium reabsorption was following a course to a considerable degree independent of the glomerular filtration rate At the points A B and C the plasma sodium concentration and the filtration rate and therefore the filtered load all have essentially the same magnitude Yet the rate of excretion of sodium at these points of equal load progressively diminishes from A to C

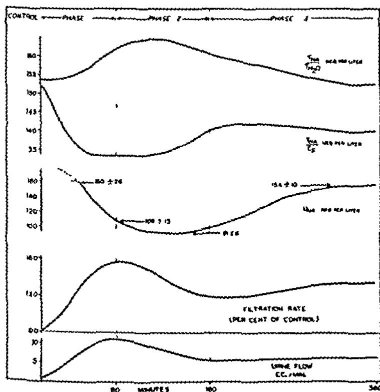


FIGURE 40

Urine sodium concentration (U_{Na}) sodium reabsorption per unit of glomerular filtrate ($\frac{T_{Na}}{\alpha C_F}$) and nominal concentration of sodium in the total reabsorbate ($\frac{T_{Na}}{T_{H_2O}}$) calculated from the mean values f r the six longest experiment by the same procedure as for the curves in Figure 37 Urine flow and filtration rate are repeated from Figure 37 for comparison Numbers are means and mean deviations from the m

Reciprocally the rate of reabsorption of sodium at constant load was increasing

What is probably a consequence of increasing tubular reabsorption of sodium is the interesting observation that it was usual in our experiments to detect a transitory period when the urine is hypotonic to plasma (Figure 40) The urine may have an osmotic U/P ratio as measured by freezing point as low as 0 85 Infusion of pitressin does not prevent hypotonicity

With three semi independent variables filtration rate sodium reabsorption and water reabsorption it is hardly surprising that the ratio of sodium reabsorption to filtration rate (T_{NA}/C_F) and the ratio of sodium reabsorption to water reabsorption (T_{NA}/T_{H_2O}) (Figure 40) show peculiar curves

It is hard to escape the conclusion that elevation of the filtration rate represents an important means of excreting sodium in the dog The work of Ladd and Raisz (Ladd M and Raisz L G Response of the normal dog to dietary sodium chloride *Am J Physiol* 159 149 (1949)) in our laboratory showed that 50 grams of sodium given by mouth to a 15 kilogram dog were at least 90% excreted in 20 hours The process of excretion was accompanied by a great rise in filtration rate and urine flow Furthermore this rate of salt ingestion for a week resulted in no change in weight Since the available data for man does not indicate any comparable increase in filtration rate under isotonic salt load one wonders whether a profound species difference does not exist and whether man may not rely for control of sodium excretion primarily on changing reabsorptive capacity rather than changing load

Shannon I want to comment on certain feeding experiments where the only thing measured was the weight of patients placed on high or low salt diets The patients returned to their initial weight about three days after the change in sodium load and it would appear that the time required for the patient to accommodate himself to the new situation in terms of excretory mechanisms is of that order though it depends in part upon the preceding salt intake Actually we were led to do those experiments because we observed pretty much what Dr Wesson has described here that is a five to ten percent expansion of extracellular fluid in the dog led to a five or ten percent dilution of plasma and that in turn was accompanied by increase in the glomerular filtration rate All variables returned to normal at about the same time We thought we had finally found a mechanism that was responsible for the control

of volume of extracellular fluid In setting up similar experiments in the human we were quite disappointed

I think one ought to bring out the fact that these studies were performed in what is commonly called a standard state and did not permit examination of such variables as posture mild exercise sleep etc any or all of which may have important effects on the functions we were interested in It appeared likely however that extrarenal factors were of importance The extent of these is perhaps brought out very well by some data that Dr White collected some years ago It would be interesting if he told us about it

White If one changes from standing up to lying down there is about a threefold increase in the rate of output of water and chloride a sixfold or sevenfold increase of the output of bicarbonate and about a 10 percent increase in glomerular filtration rate as measured by inulin or creatinine clearances and intermediate increases in the substances which are partly but not so nearly completely reabsorbed as is sodium This was done a long time ago we did not do sodium and potassium but sulfate and phosphate increase 30 40 or 50 percent as does urea I must ask anyone who may consult the paper in which the findings are presented (White H L Rosen I T Fischer S S and Wood G H The influence of posture on renal activity *Am J Physiol 78 185 (1926)*) to disregard our labored attempts at interpretation and consider only the data I now think of it in this way with substances which normally are almost completely reabsorbed if you bring about even a slight increase in filtration rate as on changing from standing up to lying down and if there is no change or a lesser increase in the amount reabsorbed there will be of course a great increase in the amount excreted while the substances which are intermediate as sulfate and phosphate will show intermediate degrees of change and substances which are not at all reabsorbed will show the least change These changes in outputs are the direct and immediate result of changes in filtration rate in the absence of immediate corresponding changes in the extent of tubular reabsorption They do not mean that the capacity of the tubules to reabsorb has been overwhelmed they mean simply that nothing has happened yet to inform the tubules that sodium or chloride is being excreted in excessive amounts so they continue to reabsorb the same amounts as before I cannot believe that even a doubling of the filtration rate over taxes the reabsorptive capacity of the tubules One can double the filtration rate in the dog for many days by giving certain anterior lobe preparations but this does not deplete him of salt his tubules

will soon increase their reabsorption so that the amount excreted is essentially the same as before the filtration was doubled The increases in salt output seen with pure increases in filtration rate do not last very long When a man lies down he begins to put out 3 times as much salt as while standing but so far as I know there is no evidence that one goes into any measurable negative sodium balance merely by going to bed for a week although there is negative balance for the first few hours The sequence of events is that on lying down one quickly gets to the point where the plasma sodium level drops a little bit, the tubules recognize this and start taking back a little more sodium I don t know how the tubules know, but they will reabsorb the amount they need to reabsorb to keep plasma levels about constant if they didn't, you would soon die If they fail for a short time, as they do when filtration rate is first increased, to reabsorb sodium in the proper amount, the plasma level will soon fall and that signals them, through hormonal influences or in some more direct way, to increase the amount taken back Fluctuations in filtration rate are not the primary cause of fluctuations in reabsorption Reabsorption continues unchanged for a while after filtration rate is increased it is the resultant slight drop in plasma level which soon arouses the tubules to increased reabsorption The change in plasma level need not be great, just as the change in plasma hydrogen ion concentration necessary to effect changes in respiration need not be great A further point is that such changes in plasma level in order to be effective, must be acutely produced, the rate of change may be the decisive factor The plasma sodium level today may be 4 percent higher than a week ago with no change in output, but so may the plasma hydrogen ion concentration show changes that are great from one week to the next without any change in respiratory volume, although such changes acutely produced might show great effects To summarize these remarks while it is true, in the long run that changes in filtration rate are accompanied by corresponding changes in the amount of sodium reabsorbed the changes in reabsorption are not the direct result of the changes in filtration but an indirect result brought on by the slight change in plasma level which is the direct result of changed filtration rate with temporarily unchanged reabsorption

Berliner There is one thing about this that disturbed me As I understand it Dr Wesson's hypothesis is that as long as there is any excretion of sodium in the urine there is a saturation of some mechanism

Wesson That would be our viewpoint with the qualification that excretion should be greater than in trace amounts and not caused by non reabsorbable anions

Berliner You can explain that with the hypothesis of reabsorption by two mechanisms — one varying with the amount of filtered the other with a fixed capacity Entirely aside from that it would seem to me that this would be a unique situation in renal physiology if such were the case that is if sodium were excreted only when the reabsorptive capacity is saturated The measurement of Tm falls I think in the category of what Dr Shannon calls finding out what tricks the kidney can do Actually if we consider all the various reabsorptive mechanisms characterized by measurable Tm s there is no reason to believe that any of them except possibly sulfate (Dr Pitts probably knows a lot more about this than I do) is saturated when the substance in question begins to be excreted in the urine I see no particular reason why the situation should be different in the case of sodium especially when we have to force ourselves and force the data quite a bit to make it come out that way Have you any comments on that Dr Pitts?

Pitts I am becoming convinced that I don t know anything about sodium I have been working on it for several years and I know much less than I thought I did when I started

Berliner When amino acids phosphate and even glucose appear in the urine it is usually not because the Tm s are saturated It is very strikingly true of uric acid I don t know why we should expect something different in the reabsorption of sodium whether there is a Tm for sodium or not

Bott You say that includes glucose?

Berliner Most glucose excretion occurs at those plasma glucose concentrations examined only in "titration experiments The individual who puts out a little glucose after a meal does not do so because his Tm is saturated Usually we expect people to spill glucose at a level of 175 200 mg percent and calculating from the average filtration rate and glucose Tm a normal individual should not spill until his level got to 300 Actually to saturate the glucose reabsorptive capacity you have to go up to 400 mg percent

Pitts One thing which I might add to confuse the issue somewhat is the fact that if you load a normal dog with sodium chloride by infusing a 3 5 percent solution at 10 cc per minute and measure his capacity to eliminate or expressed in the other way his capacity to absorb take out the adrenals and do the same experiment a couple weeks later the adrenalectomized animal ... ...sorb more

than the normal animal He excretes less and thus has excretory tolerance

Luetscher We heard from Dr Taggart that Tm is not sacred and fixed point that it has to stand still under all con Not only may there be variations below a certain load, but e maximum may change with the action of various stimuli on t I think that we should turn more of our thought to some factors which somehow got down at the end of the list of va for our consideration Endocrine secretions are known to inf renal functions and it seems almost certain that there are ch in endocrine function during experiments which involve str of the types described We still need a great deal of fundam data, and we need better methods for the assay of endocrine f tion so that we can measure these factors which can shift the and remove the fixed point of calculations

Pitts As the Chairman of this group I want to thank all of y for your participation in the conference Your contributions to have made it a success